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Aims and Scope

Journal of Pharmaceutical Negative Results (www.pnrjournal.com) [ISSN: Print -0976-9234, Online - 2229-7723] – (An official publication of Association of Indian pharmacist-AIP, Published by ResearchTrentz). The journal is a peer-reviewed journal developed to publish original, innovative and novel research articles resulting in negative results. This peer-reviewed scientific journal publishes a theoretical and empirical paper that reports the negative findings and research failures in pharmaceutical field. Submissions should have a negative focus, which means the outputs of research yielded in negative results are being given more preference. All theoretical and methodological perspectives are welcomed. We also encourage the submission of short papers/communications presenting counter-examples to usually accepted conjectures or to published papers. This Journal is a biannual publication.

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Contents

Absence of antidiabetic activity in some novel thiazolidinone derivatives

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A B S T R A C T

Aim: *It was aimed to synthesise some novel thiazolidinone derivatives and assess them for antidiabetic activity. Material and Methods: A series of substituted 5-ethylidene2-(phenylimino) thiazolidin-4-ones were prepared by using phenylthiourea (I) as a starting material. Phenylthiourea on reaction with ethylchloroacetate, in the presence of ethanol and fused sodium acetate, gave 2-(phenylimino) thiazolidin-4-one (II), and 2-(phenylimino) thiazolidin-4-one on further reaction with substituted benzaldehyde gave substituted 5-ethylidene-2- (phenylimino) thiazolidin-4-one (III – XVIII). The synthesized compounds were authenticated on the basis of elemental analysis, IR, 1H NMR, and Mass spectral analysis and some of the compounds were selected on the basis of a literature review, to evaluate them for their antidiabetic activity. Results and Conclusion: All The tested compounds 5-(4-fluorobenzylidene)-2-(phenylimino) thiazolidin-4-on (VII) and 5-(4-Methylbenzylidene)-2-(phenylimino)thiazolidin-4-one (X), 5-(2, 4-dinitrobenzylidene)-2-(phenylamino) thiazolidin-4-one (XVII) were found to be ineffective in lowering the blood glucose level.*

Key words: Heterocyclic, substitution, synthesis, thiazole, thiazolidinone

INTRODUCTION:

Diabetes belongs to a group of metabolic disorders in which the body is not able to produce sufficient amount of insulin or the body cells do not respond to the insulin that is produced, leading to symptoms such as increased urination, extreme thirst, and unexplained weight loss. In its most severe form, ketoacidosis or a non-kenotic hyperosmolar state may develop and lead to stupor, coma, and in absence of effective treatment, often death.[1] Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central the nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.[2] Diabetes is mainly divided into three types Type I or Insulin-dependent diabetes mellitus (IDDM), which includes those cases that occur due to an autoimmune process, as well as those with beta-cell destruction, and in those who are prone to ketoacidosis for which neither an etiology nor a pathogenesis is known (idiopathic). It does not include those forms of beta-cell destruction or failure to which specific causes can be assigned (e.g. cystic f ibrosis, mitochondrial defects, and the like). Type II or non-insulin dependent diabetes mellitus (NIDDM) includes the common form of diabetes, which results from a defect in insulin secretion, almost with a

major contribution from insulin resistance.[3] Type III gestational diabetes is a hyperglycemic condition that occurs due to carbohydrate intolerance, with onset or first recognition during pregnancy. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy. Individuals at high risk for gestational diabetes include older women, those with a previous history of glucose intolerance. As far as the antidiabetic activity is concerned thiazolidinone has been reported to possess diversified activities including hypoglycemic action. [4] Drugs like Pioglitazone and Rosiglitazone contain a heterocyclic thiazolidinone ring, which plays an important role in antidiabetic activity.[5]

Chemistry

The chemistry of a thiazolidin-4-one ring system is of considerable interest as it is the core structure in various synthetic pharmaceuticals, which display a broad spectrum of biological activities. These are heterocyclic compounds that have an atom of sulfur at position 1, an atom of nitrogen at position 3, and a carbonyl group at position 4.[6] Heterocyclic compounds are cyclic compounds with at least two different elements as ring member atoms.[7] They are the counter parts of homocyclic compounds, which have ring atoms from the same element. Although heterocyclic compounds may be inorganic, most of them contain at least one carbon atom, and one or more atoms of elements other than carbon within the ring structure, such as sulfur, oxygen or nitrogen.[8] Thiazolidinone, a saturated form of thiazole, with a carbonyl group on the fourth carbon, has been considered to have a large number of biological activities [Figure 1]. Substitution can be done at positions 2, 3, and 5, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in position 2 [Figure 2]. The carbonyl group present in the moiety is

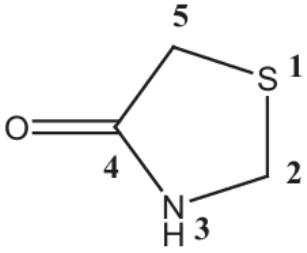
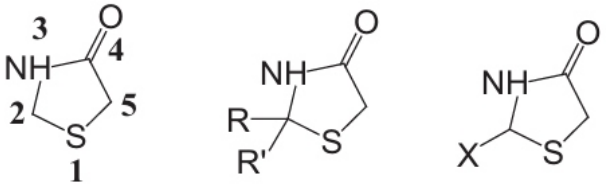


Figure 1: Structure of 4-Thiazolidinone



X = [= O, = S, NR, NN = CRR']
R, R' = Cl, F, C_5H_5Cl

Figure 2: Various Thiazolidinone rings and their substituents

highly unreactive. The tetrahydro derivative of thiazole is known as thiazolidine and the oxo-derivative of thiazolidine is known as thiazolidinone. The 3-unsubstituted thiazolidinones are usually solids, but the attachment of an alkyl group to the nitrogen lowers the melting point. The thiazolidinones that do not contain aryl or higher alkyl substituents are slightly soluble in water.[9] 4-Thiazolidinone derivatives are known to possess antibacterial,[10,11] antifungal,[12,13] antiviral,[14,15] antituberculosis,[16] and anti-convulsant[17] properties. 4-Thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B, which is a precursor, which acts during the biosynthesis of peptidoglycan. [18] 4-Thiazolidinones of diflunisal have been found to be dual acting antimicrobial / antituberculosis agents possessing anti-inflammatory properties via the active metabolite, diflunisal, and are active against pain and inflammatory events, due to the cell damage arising from tuberculosis and the accompanying infectious diseases.[19] 2, 3-disubstituted analogs of Thiazolidinone have proved to be predominantly effective non-nucleoside HIV reverse-transcriptase inhibitors. [20] It was observed that reaction with cyclizing reagents like α-halocarbonyl compounds such as, ClCH2 COCl, BrCH2 COCl, BrCH2 COOEt, and ClCH2 COCH2 COOEt in boiling ethanol, with fused sodium acetate, have better biological profiles and a better yield.[21,22] The thiazolidin-4one ring system also occurs in nature as asactithiazic acid, ((-)2-(5-carboxypentyl)thiazolidin-4-one), which is isolated from the Streptomyces strains.[22]

MATERIALS AND METHODS

All the chemicals and reagents were obtained from Sigma (Germany) and CDH (India) and were recrystallized / redistilled as necessary. The melting points were determined by the open capillary tube method. The purity of the compounds was checked on thin layer chromatography (TLC) plates, which were precoated with silica gel G using solvent system toluene : ethyl acetate : formic acid (5 : 4 : 1). The spots were located under iodine vapors and ultraviolet (UV) light. Infrared (IR) spectra were recorded using KBr on Fourier transform infrared (FTIR) Shimadzu 8400S IR spectrophotometer (Japan). A JEOL AL300 FTNMR 300 MHz spectrometer was used to acquire High Resolution Nuclear Magnetic Resonance (1HNMR) spectra with Acetone as the solvent and tetramethylsilane (TMS) as the internal standard. Chemical shift values are expressed in ppm. Mass spectra were obtained using a Kratos-AEI MS902S instrument. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (CDRI, Lucknow). The results of the elemental analysis (C, N, and S) were within ± 0.4% of the calculated amounts.

Synthesis

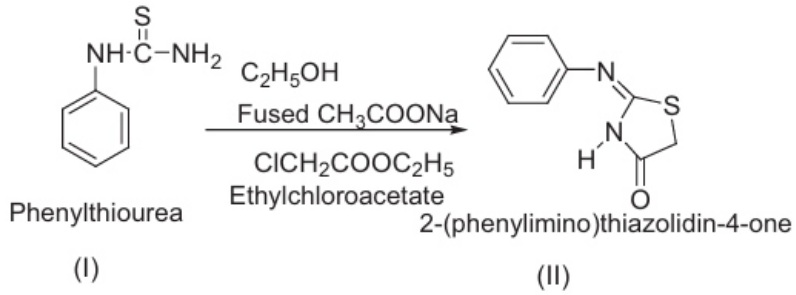
Step 1

General Procedure for synthesis of

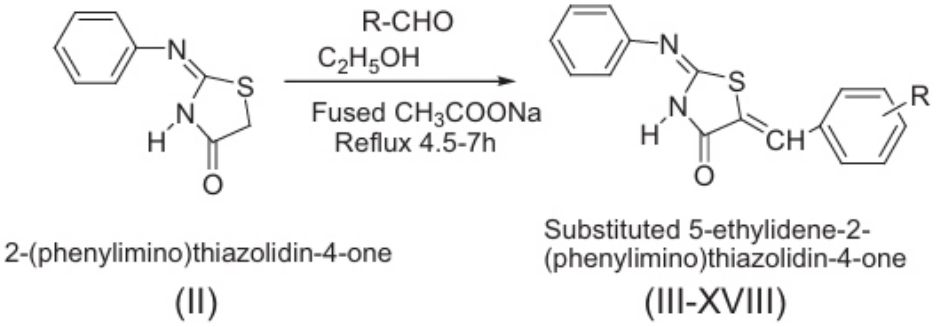
2-phenyliminothiazolidin-4-one (II)

Phenyl thiourea (I) 8 g (0.04 moles) was dissolved in 16.45 ml ethanol. The resulting mixture was refluxed with fused sodium acetate 4.31g (0.052 moles) and ethylchloroacetate 6.46 g (5.65 ml) for four hours.

The reaction mixture was then poured into water.



Scheme 1: Synthesis of 2-(phenylimino) thiazolidin-4-one



Scheme 2: Synthesis of substituted 5-ethylidene-2-(phenylimino) thiazolidin-4-ones

The reaction mixture was kept overnight for complete precipitation. The precipitate obtained was filtered and dried at room temperature. Further it was recrystallized with ethanol [Scheme 1].

2-phenyliminothiazolidin-4-one (II)

Yield: 80.79% (solid); m.p: 175–177°C; Rf value (T: E: F; 5: 4: 1): 0.75, IR (KBr): 3415(N-H), 1745(C = O), 1610 (C = N)cm−1, 1H NMR (Acetoned-6, 300 MHz): d = 3.21 (s, 2H, CH2), 6.98 – 7.34 (m, 5 H, phenyl), 11.82 (s, 1H, NH), MS m / z: 192 (M+), Anal. Calcd for C9 H8 N2 OS: C, 55.26; N, 14.10; S, 16.60 [Table 1].

Step-2

2.2.2 General Procedure for the preparation of substituted Thiazolidinone Derivatives (III–XVIII)

2-phenyliminothiazolidin-4-one (II) (0.01 mole) was reacted with different aromatic aldehydes (0.01 mole) with fused sodium acetate (0.01mole) in ethanol (8 ml) for six to seven hours. The reaction mixture was then cooled to room temperature, poured into ice cold water and kept overnight. The precipitate obtained was filtered and washed with water to remove the aldehyde that had not reacted. Further this precipitate was dried at room temperature. The product obtained was recrystallized from dimethyl formamide [Scheme II].

Table 1: Physical property of synthesized compound (II)

Compound	R	Molecular formula	M. Wt	Rf Value	%Yield
II	H	C9H8N20S	192.24	0.75	80.79

Solvent system used: Toluene : Ethyl acetate : Formic acid (5:4:1)

Table 2: Physical properties of synthesized compounds (III–XVIII)

Compound code	R	Molecular Formula	Mol. Wt.	m.p.(°C)	Yield (%)	R^f
III	Benzaldehyde	$C_6H_{12}N_2OS$	280.34	258 – 260	68.80	0.71
IV	2,4dichlorobenzaldehyde	$C_{16}H_{10}C_{l2}N_2S$	349.23	186 – 188	87.90	0.67
V	2-Nitrobenzaldehyde	$C_{16}H_{11}N_3O_3S$	325.34	297 – 299	41.20	0.76
VI	4-Methoxybenzaldehyde	$C_{17}H_{14}N_2O_2S$	310.37	195 – 197	93.75	0.63
VII	4-Fluorobenzaldehyde	$C_{16}H_{11}FN_2OS$	298.33	272 – 274	66.00	0.68
VIII	3,4,5trimethoxybenzaldehyde	$C_{19}H_{18}N_2O_4S$	370.42	210 – 212	62.16	0.70
IX	4-chlorobenzaldehyde	$C_{16}H_{11}ClN_2OS$	314.79	285 – 287	76.19	0.80
X	4-Methylbenzaldehyde	$C_{17}H_{14}N_2OS$	294.37	202 – 204	62.00	0.72
XI	5-Methylsalicyldehyde	$C_{17}H_{14}N_2O_2S$	310.37	239 – 241	71.10	0.77
XII	4-Nitrobenzaldehyde	$C_{16}H_{11}N_3O_3S$	325.34	320 – 322	54.50	0.73
XIII	2-chlorobenzaldehyde	$C_{16}H_{11}ClN_2OS$	314.79	200 – 202	82.80	0.65
XIV	Anisaldehyde	$C_{16}H_{12}N_2O_2S$	296.34	280 – 282	61.00	0.78
XV	2,4difluorobenzaldehyde	$C_{16}H_{10}F_2N_2OS$	316.33	240 – 242	60.50	0.62
XIV	2-Fluorobenzaldehyde	$C_{16}H_{11}FN_2OS$	298.33	260 – 262	82.20	0.76
XVII	2,4dinitrobenzaldehyde	C\H\N$_4$O$_5$S	370.34	242 – 244	51.40	0.74
XVIII	2-Bromobenzaldehyde	$C_{16}H_{11}BrN_2OS$	359.24	270 – 272	78.20	0.60

TLC Solvent system used: Toluene: Ethyl acetate: Formic acid (5:4:1)

Spectral data of compounds

5-Benzyliden-2-(phenylimino) thiazolidin-4-ones (III)

Yield: 68.80% (solid); m.p: 258–260˚C, Rf value (T: E: F; 5:4:1): 0.71, IR (KBr) cm-1: 3251.76 (N-H); 30251.76 (Ar C-H); 1677.95 (C = O); 1637.45 (C = N). 1H NMR (Acetone-d6, 300 MHz, d ppm); 6.9 – 7.8 (m, 10H, phenyl and benzylidene); 7.35 (s, 1H, C = CH); 7.8 (s, 1H, NH), Mass (m / z) 495.0 (M+). Anal. Calcd (C6H12N2OS): C, 67.20; N, 8.90; S, 11.40.

5-(2, 4-dichlorobenzylidene)-2-(phenylimino) thiazolidin-4-one (IV)

Yield 87.90% (solid); mp 186 – 188˚C, Rf value (T: E: F; 5: 4: 1): 0.67, IR (KBr) cm-1: 3461.47 (N-H); 3037.21 (Ar C-H), 2953.56 (aliphatic C-H), 1671.62 (C = O), 1H NMR (Acetone-d6,300 MHz, d ppm) 6.99 – 7.20 (m,10H, phenyl and benzylidene); 7.60 (s,1H,C = CH); 8.2 (s, 1H, NH), Mass (m / z) 347.98 (M+). Anal. Calcd (C16 H10 Cl2 N2 OS): C, 55.0; N, 8.00; S, 9.10 [Table 2].

5-(2-nitrobenzylidene)-2-(phenylimino) thiazolidin-4one (V)

Yield: 41.20% (solid); m.p: 297-300˚C, Rf value (T: E: F; 5:4:1): 0.76, IR (KBr) cm-1: 3420 (N-H); 3031 (Ar C-H); 1660 (C = O); 1600 (C = N); 1492 (C-NO2); 1H NMR (Acetone-d6,300 MHz, d ppm): 6.90 – 7.32 (m, 5H,phenyl); 7.60 (s,1H,C = CH); 7.70-8.24 (m, 4H, 3-nitrobenzylidene); 8.7 (s, 1H, NH), Mass (m / z) 325.04 (M+). Anal. Calcd C16 H11 N3 O3 S: C, 59.06; N, 12.90; S, 9.82 [Table 2].

5-(4-methoxybenzylidene)-2-(phenylamino) thiazolidin-4-one (VI)

Yield: 93.75% (solid); m.p: 195-197˚C, Rf value (T: E: F; 5:4:1): 0.63, IR (KBr) cm-1: 1674.10 (C = 0), 1384.79 (CH = CH), 1244.00 (N-H), 1H NMR (Acetone-d6, 300 MHz, d ppm): 7.34 – 7.60 (m, 9H, phenyl and benzylidene); 7.39 (m, 1H, C = CH); 7.8 (s, 1H, NH), Mass (m / z) 386.1 (M+). Anal. Calcd (C17 H14 N2 O2 S): Calcd C 65.30, N 8.97, S, 10.25 [Table 2].

5-(4-fluorobenzylidene)-2-(phenylimino) thiazolidin4-on (VII)

Yield: 66.00%(solid); m.p: 272 – 274˚C, Rf value(T: E: F; 5:4:1): 0.68, IR (KBr) cm-1: 3467 (N-H); 3050 (Ar C-H); 1680 (C = 0); 1600 (C = N);1032 (C-F); 1H NMR (Acetone-d6, 300 MHz, d ppm): 6.89–7.34 (m, 5H, phenyl); 7.13 (d, 2H, benzylidine); 6.91 (d, 2H, benzylidine), 7.39 (s, 1H,C = CH); 7.73 (s, 1H, NH), Mass (m / z) 374.1 (M+). Anal. Calcd (C16 H11 Fn2 OS): C, 64.40; N, 9.35; S, 10.73 [Table 2].

5-(3, 4, 5-trimethoxybenzylidene)-2-(phenylimino) thiazolidin-4-one (VIII)

E: F; 5: 4: 1): 0.70; IR (KBr) cm-1: 1660.23 (C = O), 1180.86 (CH = CH), 1220.24 (CH = CH), 1H NMR (Acetone-d6, 300 MHz, d ppm): 3.82 (s, 9H, OCH3); 8.14 (s, 1H, C = CH); 8.01 (s, 1H, NH) Mass (m / z) 390.09 (M+). Anal. Calcd (C19 H18 N2 O4 S): C, 61.61; N, 7.54; S, 8.60 [Table 2].

5-(4-chlorobenzylidene)-2-(phenylimino) thiazolidin4-one (IX)

Yield: 76.19%, m.p: 285–287˚C; Rf value (T: E: F; 5: 4: 1): 0.80; IR (KBr) cm-1: 1633.59 (C = O),1244.00 (CH = = CH),1091.63 (N = H); 1H NMR (Acetone-d6, 300 MHz, d ppm): 6.9–7.2 (m,5H,phenyl); 7.20 (d, 2H, benzylidene); 7.22 (d, 2H, benzylidene); 6.70 (s, 1H, C = CH); 8.38 (s, 1H, NH); Mass (m / z) 390.1 (M+). Anal. Calcd (C16 H11 ClN2 OS): C, 61.02; N, 8.70; S, 10.19 [Table 2].

5-(4-Methylbenzylidene)-2-(phenylimino) thiazolidin 4-one (X)

Yield: 62.00%, m.p: 202–204, Rf value (T: E: F; 5:4:1): 0.72, IR (KBr) cm-1: 1550.94 (CH2-CH2) ,1770.60 (C = O), 3038 (Ar C–H), 2954 (aliphatic C–H) , 1337.22 (N-CH2), 1H NMR (Acetone-d6, 300 MHz, d ppm): 2.08 (s, 3H, CH3); 6.89–7.23 (m, 5H, phenyl); 7.36 (d, 2H, benzylidene); 7.51 (d, 2H, benzylidene); 7.76 (s, 1H, C = CH); 7.8 (s, 1H, NH). Mass (m / z) 294 (M+) [Table 2].

5-(2-hydroxy-5-methylbenzylidene)-2-(phenylimino) thiazolidin-4-one (XI)

Yield: 71.10%, m.p: 239-241, Rf value (T: E: F; 5:4:1): 0.77, IR (KBr) cm-1: 1530.94 (CH2-CH2), 1670.60 (C = O), 3565 (O–H), 3029 (Ar C–H), 1357.22 (N-CH2). 1H NMR (Acetone-d6, 300 MHz, d ppm): 6.71–7.31 (m, 9H, phenyl and benzylidene); 7.69 (s, 1H, C = CH); 8.40 (s, 1H, NH); 9.85 (s, 1H, OH). Mass (m / z) 296 (M+) [Table 2].

5-(4-nitrobenzylidene)-2-(phenylimino) thiazolidin-4one (XII)

Yield: 54.50%, m.p: 320–322, Rf value (T: E: F; 5:4:1): 0.73, IR (KBr) cm-1: 1515.94 (CH2-CH2) ,1660.60 (C = O), 1346.22 (N-CH2) ,1H NMR (Acetone-d6, 300 MHz, d ppm): 6.99–7.04 (m, 5H, phenyl; 7.63 (s, 1H, C = CH); 7.58 (m, 2H, 4-nitrobenzylidene); 8.21 (m, 2H, 4-nitrobenzylidene); 8.44 (s, 1H, NH), Mass (m / z) 586.1 (M+). Anal. Calcd. (C16 H11 N3 O3 S): C, 59.07; N, 12.90; S, 9.80 [Table 2].

5-(2-chlorobenzylidene)-2-(phenylimino) thiazolidin 4-one (XIII)

Yield: 82.80%, m.p: 200 – 202, Rf value (T: E: F;5:4:1): 0.65, IR (KBr) cm-1: 1520 (CH2-CH2), 1670 (C = O), 1070 (N-CH2) , 1H NMR (Acetone-d6,300 MHz, d ppm): 6.99 – 7.0 (m, 5H, phenyl); 7.20 (s, 1H, C = CH); 7.00 – 7.20 (m, 4H, chlorobenzylidene); 8.2 (s, 1H, NH) Mass spectra (m / z) 314.02 (M+). Anal. Calcd (C16 H11 ClN2 OS): C, 61.04; N, 8.80; S, 10.19 [Table 2]

5-(phenoxymethylene)-2-(phenylimino) thiazolidin4-one (XIV)

Yield: 61.00%, m.p: 280-282, Rf value (T: E: F; 5:4:1): 0.78, IR (KBr) cm-1: 1670.24 (C = O), 1240.14 (CH = CH), 1176.50 (N = H); 1H NMR (Acetone-d6, 300 MHz, d ppm): 6.91 – 7.01 (m, 5H, phenyl) 6.89 (s, 1H, C = CH); 6.89-7.01(Ar C-H benzylidene); 7.36 (s, 1H, NH), Mass (m / z) 525.0 (M+). Anal. Calcd (C16 H12 N2 O2 S): C, 64.80; N, 9.40; S, 10.80 [Table 2].

5-(2, 4-difluorobenzylidene)-2-(phenylimino) thiazolidin-4-one (XV)

Yield: 60.50%, m.p: 240-242, Rf value (T:E:F; 5:4:1): 0.62; IR (KBr) cm-1: 3468 (N-H); 3025 (Ar C-H); 1674 (C = O); 1608 (C = N); 1038 (C-F); 1H NMR (Acetone-d6,300 MHz, d ppm): 6.99-7.00 (m, 5H, phenyl); 7.00 (s, 1H, C = CH); 8.2 (s, 1H, NH); 6.60 7.25 (Ar C-H benzylidene); Mass (m / z)

316.04 (M+). Anal. Calcd (C16 H10 F2 N2 OS): C, 6.072; N, 8.80; S, 10.10 [Table 2].

5-(2-fluorobenzylidene)-2-(phenylimino) thiazolidin4-one (XVI)

Yield: 82.20% m.p: 260 – 262, Rf value (T: E: F; 5:4:1): 0.76, IR (KBr) cm-1: 3252.43 (N-H), 1616.94 (C = N), 1548.60 (C = C), 1513.33, 1447.40, and 1048.88; 1H NMR (Acetone-d6, 300 MHz, d ppm): 6.99-7.00 (m, 5H, phenyl); 7.00 (s, 1H, C = CH); 8.2 (s,1H, NH); 6.80 – 7.25 (Ar C-H benzylidene); Mass (m / z) 296.06 (M+). Anal. Calcd (C16 H11 Fn2 OS): C, 64.40; N, 9.30; S, 10.74 [Table 2].

5-(2, 4-dinitrobenzylidene)-2-(phenylimino) thiazolidin-4-one (XVII)

Yield: 51.40% m.p: 242 – 244, Rf value (T: E: F; 5:4:1): 0.74, IR (KBr) cm-1: 1664 (C = O), 1160 (C = CH), 670 (Ar str), 1340 (NO2 str); 1608 (C = N); 1H NMR (Acetone-d6, 300 MHz, d ppm): 6.99-7.00 (m, 5H, phenyl); 7.32 (s, 1H, C = CH); 8.2 (s, 1H, NH); 7.80–9.00 (Ar C-H benzylidene) Mass (m / z) 370.02 (M+). Anal. Calcd (C16 H10 N4 O5 S): C, 51.89; N, 15.12; S, 8.60 [Table 2].

5-(2-bromobenzylidene)-2-(phenylimino) thiazolidin4-one (XVIII)

Yield: 78.20% m.p: 270–272, Rf value (T: E: F; 5:4:1): 0.60, IR (KBr) cm-1: 1384.79 (C = C), 756.04 (C-H Ar), 686.61 (C-Br), 1H NMR (Acetone-d6,300 MHz, d ppm): 6.99 – 7.00 (m, 5H, phenyl); 7.37 (s,1H, C = CH); 8.0 (s, 1H, NH); 7.34–7.42 (Ar C-H benzylidene), Mass (m / z) 572.9 (M+). Anal. Calcd. (C16 H11 BrN2 OS): C, 53.48; N, 7.80; S, 8.90 [Table 2]. Antidiabetic activity Animal Albino-Swiss rats weighing (150 – 200 g) were used for studying in-vivo antidiabetic activity. Animals were maintained under standard laboratory conditions (24 ± 2°C; relative humidity 60 – 70%). A study protocol was approved by the Institutional Animal

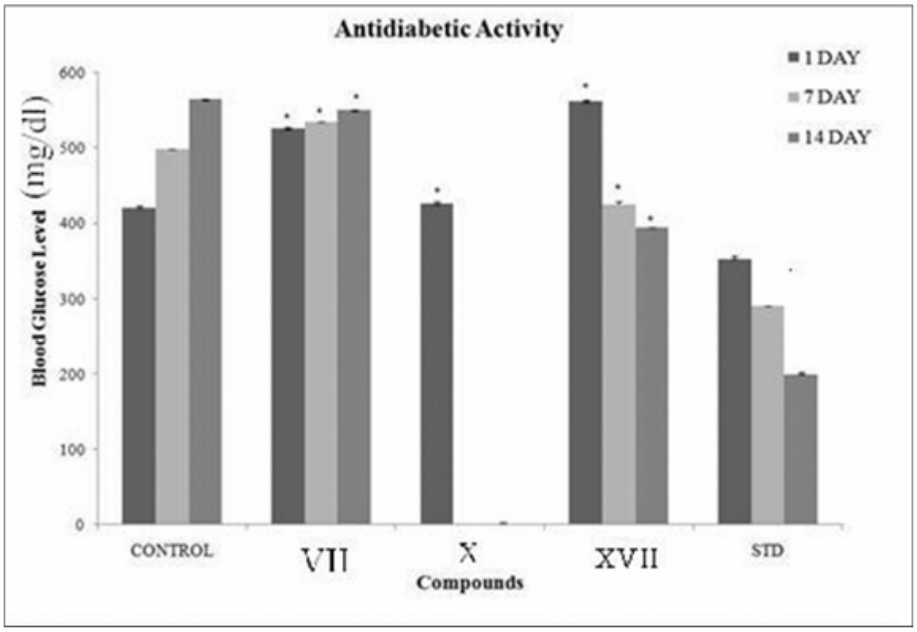


Figure 3: Antidiabetic Activity of Synthesized Compounds at a Dose of (200 mg / kg) in albino mice, All the values are expressed as Mean ± S.E.M (n = 6). *P ≤ 0.05 and **P ≤ 0.01, ***P ≤ 0.001 indicates the level of significance when compared with the control

Table 3: Antidiabetic activity of the synthesized compound

Compounds	Average Serum Glucose Level (mg / dL)		
	First day	Seventh day	Fourteenth day
Control	421 ± 1.52	498 ± 0.57	564 ± 1.20
Standard	353 ± 1.15	291 ± 0.57	472 ± 0.88
VII	526 ± 2.47	534 ± 3.53	550 ± 3.53
X	426 ± 1.31	-	-
XVII	561 ± 3.25	425 ± 2.50	395 ± 2.50

All values are expressed in Mean ± SEM of six animals in each group. (-) indicates that the animals died during the experiment.

Ethics Committee for the Purpose of Control and Supervision of Experiments on Animals (IAEC, Approval No.711 / 02 / a / CPCSEA) before the experiment. Albino-Swiss rats from the Laboratory Animal House Section, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut, were used in the study. The animals were kept in polypropylene cages and maintained on balanced ration with free access to clean drinking water. Induction of diabetes mellitus Streptozotocin (STZ) was obtained from sigma chemicals (USA). STZ was dissolved in cold 0.01 M citrate buffer, pH 4.5 and prepared freshly for immediate use. The animals were fasted for 20 hours and then the STZ injection was given intraperitoneally at a dose of 60 mg / kg. The blood glucose concentration was measured on the first day, seventh day, and fourteenth day, with the help of a glucometer, by using a blood sample from tail vein. Experimental groups and protocol The animals were divided into standard, test, and control. The test drug was suspended in 1% Na-CMC (Na-Carboxymethyl cellulose) and administered at a dose of 200 mg / kg orally. Subsequently, the serum glucose level was determined and is reported in Table 3 and the graphical data in Figure 3.

RESULTS

The structures of the synthesized compounds were confirmed by IR spectra, 1HNMR spectral analysis, and mass and elemental analysis. The IR spectra exhibited some characteristic bands due to = C-H str. (3100 – 3000 cm/1), C = C str. (1635 – 1495 cm/1), C-H bending (900 – 860 cm/1), C-H bending (substituted aryl (840 – 800 cm/1), C-Cl str. (750 – 700 cm/1), C-F str. (1100 – 1000 cm/1), C-S-C str. (700 – 600 cm/1), C = N (ring) (1650 – 1580 cm/1) stretching vibration band, and C = O (1674 cm/1, 4-thiazolidinone moiety). In the 1H NMR spectra the signals appeared between d 5.1 and 6.1 indicating the presence of thiazolidinone.

CONCLUSION

The tested compounds 5-(4-fluorobenzylidene)2-(phenylimino) thiazolidin-4-on (VII) and 5-(4-Methylbenzylidene)-2-(phenylimino)thiazolidin4-one (X), 5-(2, 4-dinitrobenzylidene)-2-(phenylamino) thiazolidin-4-one (XVII) were found to be ineffective in lowering the blood glucose level.

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REFERENCES

1. Kuzuya T, Matsuda A. Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. Diabetes Care 1997;20:219-20.

2. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. Diabet Med 1998;15:539-53.

3. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ 1994;308:1323-8.

4. Wahl PW, Savage PJ, Psaty BM, Orchard TJ, Robbins JA, Tracy RP, et al. Diabetes in older adults: Comparison of 1997 American Diabetes Association classification of diabetes mellitus with 1985 WHO classification. Lancet 1998;352:1012-5.

5. Firke SD; Firake BM, Chaudhari RY, Patil VR. Synthetic and Pharmacological Evaluation of Some Pyridine Containing Thiazolidinones. Asian J Res Chem 2009;2:345-56.

6. Wilson, Gisvold. Text Book of Organic Medicinal and Pharmaceutical Chemistry. Philadelphia: Lippincott-Raven publishers; 2003. p. 1-3.

7. Eicher T, Hauptmann S. The chemistry of heterocycles: Structure, reaction, synthesis, and applications. Hoboken, New Jersey: WileyVCH; 2003. p. 207-9.

8. Gilchrist TL. Organic synthesis of heterocyclic chemistry. Sweden: Swedish Press Publication;

1963. p. 203-7.

9. Brown FC. R. 4-thiazolidinone. Chem Rev 1961;61:463-7.

10. Kucukguzel SG, Oruc EE, Rollas S, Şahin F, Ozbek A. Synthesis, characterisation and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds. Eur J Med Chem 2002;37:197-206.

11. Ballell L, Field RA, Duncan K, Young RJ. New Small-Molecule Synthetic Antimycobacterials. Antimicrob Agents Chemother 2005;49:2153-63.

12. Cesur N, Cesur Z, Ergenc N, Uzun M, Kiraz, M, Kasımoglu O, et al. "New Acylthiosemicarbasides, Thiazolidinones, and 1,3,4-Oxadiazoles as Possible Anticonvulsants. Arch Pharm 1994;327:271-2.

13. Vartale SP, Pawde AV, Halikar NK, Kalyankar ND, Pawar YD. Synthesis, Characterization and antimicrobial activity of 6 / 7-Substutited Quinolines-4-thiazolidinones. Res. J. Pharm. Biol. Chem Sci. 2010;1:1061-7.

14. Yenamandra S, Prabhakar V, Raja Solomon, Manish K, Katti SB. QSAR Studies on Thiazolidines: A Biologically Privileged Scaffold. Heterocyclic Chem 2006;4:345-6.

15. Rao A, Balzarini J, Carbone A, Chimirri A, De Clercq E, Luca LD, et al. 2-(2,6-Dihalophenyl)-3-(pyrimidin-2-yl)-1,3-thiazolidin-4-ones as nonnucleoside HIV-1 reverse transcriptase inhibitors. Farmaco 2004;59:33-9.

16. Babaoglu K, Page MA, Jones VC, McNeil MR, Dong C, Naismith JH. Novel inhibitors of an emerging target in Mycobacterium tuberculosis; substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. Bioorg Med Chem Lett 2003;13:3227-30.

17. Srivastava SK, Srivastava S, Srivastava SD. Synthesis of New Carbazolylthiadiazol-2-oxo-azetidines: Anti-microbial, Anti-convulsant and Antiinflammatory Agents. Indian J Chem 1999;38:183-7.

18. Andres CJ, Bronson JJ, D Andrea SV, Deshpande MS, Falk PJ. GrantYoung KA. 4-Thiazolidinones: Novel inhibitors of the bacterial enzyme MurB. Bioorg Med Chem Lett 2000;10:715-7.

19. Kuçukguzel G, Kocatepe A, De Clercq E, Şahin F, Gulluce M. Synthesis and biological activity of 4-thiazolidinones, thiosemicarbazides derived from diflunisal hydrazide. Eur J Med Chem 2006;41:353-9.

20. Barreca ML, Chimirri A, De Luca L, Monforte AM, Monforte P, Rao A, et al. Anti-HIV agents: Design and discovery of new potent RT inhibitors. Bioorg Med Chem Lett 2001;11:1793-8.

21. Aridoss G, Amirthaganesan S, Kim MS, Kim JT, Jeong YT. Synthesis, spectral and biological evaluation of some new thiazolidinones and thiazoles based on t-3-alkyl-r-2, c-6s-diarylpiperidin-4-ones. Eur J Med Chem 2009;44:4199-210.

22. Kato T, Ozaki T, Tamura K. Novel calcium antagonists with both calcium overload inhibition and

antioxidant. J Med Chem 1999;42:3134-42.

Lack of human immunodeficiency virus-1 integrase inhibitory activity of novel 3a, 4, 7, 7a-tetrahydro-1H-isoindole-1,3 (2H)-dione derivatives

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ABSTRACT

Background: *Majority of reported integrase (IN) inhibitors had an important structural feature, i.e., 1,3-diketo functional group. It plays a vital role in IN inhibition by the formation of chelating triod with Mg+2 ions. Materials and Methods: A novel series of fifteen 3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-(substituted phenyl) propanamide 4(a-o) analogs were synthesized by reacting the corresponding 3-chloro-N-(substituted phenyl) propanamides 2(a-o) with 3a, 4,7,7a-tetrahydro-1H-isoindole-1,3 (2H)-dione (3) in acetonitrile medium in the presence of potassium carbonate. Various substituted 3-chloro-N-(substituted phenyl) propanamides 2(a-o) were synthesized by treating appropriate substituted anilines 1(a-o) with 3-chloro propionyl chloride in dichloromethane as solvent in the presence of triethylamine as base. The synthesized compounds have been characterized on the basis of fourier transform infrared spectrophotometerproton nuclear magnetic resonance spectrophotometer,1H NMR, Mass spectral and Elemental Analysis. Results: All the synthesized compounds were evaluated for their human immunodeficiency virus (HIV)-1 INinhibitory activity. However, unlike other anti-IN agents, none of these molecules showed inhibition of either 3' processing, and strand transfer reactions of HIV-1 IN.*

Key words: Acquired immune deficiency syndrome, highly active anti-retroviral therapy, human immunodeficiency virus-1 integrase, tetrahydrophthalimide

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is one of the major infective diseases caused by the human immunodeficiency virus (HIV). AIDS is one of the major leading causes to death. According to united nations programme on AIDS united nations programme on AIDS (UNAIDS)-2012 reports, 33 million people living with AIDS and 1.7 million people died in the year 2011.[1] Number of deaths due to the HIV infection is decreasing because of availability of highly active anti-retroviral therapy (HAART). [2]

HAART includes two nucleotide or nucleoside reverse transcriptase inhibitors and one Protease Inhibitor or one non-nucleoside reverse transcriptase inhibitor. Even though, HAART decreases viral loads, but unable to eradicate the HIV-1 virus completely from infected patients. Hence, lifelong HAART therapy is required for infected individuals because of the chronic nature of HIV-1

infection.[3] Severe adverse effects from long-term HAART medication and the rapid development of resistance to available drugs creates emergency to develop the potent and safe drugs active against alternative targets in the HIV-1 replication process.[4]

HIV integrase (IN) is one of the essential enzymes, which play a key role in HIV lifecycle along with Reverse Transcriptase and Proteases.[5] IN mediates important reactions such as, assembly of a stable nucleoprotein complex with viral deoxyribonucleic acid (DNA) sequences, cleavage of two nucleotides from both 3′-ends of the proviral DNA, and covalent joining of 3′-processed proviral DNA with host DNA.[6,7] These unique catalytic properties make HIV IN as one of the attractive targets

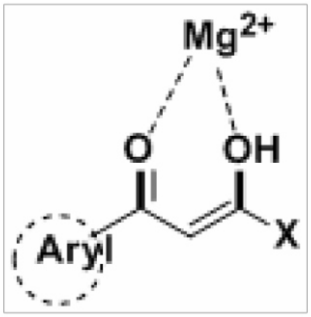


Figure 1: 1,3 diketo functional group chelating triod complex with Mg2+ ion

for development of new anti-HIV drugs. However, development of clinically effective IN inhibitors is challenging task. Majority of anti-IN agents possess 1,3-diketo functional group as common structural feature [Figure 1]. HIV-1 IN inhibitors form chelating tripod with Mg2 + ion present in IN enzyme with this 1, 3-diketo functionality and there by inhibit HIV-1 IN catalyzed 3′-processing and strand transfer (ST) reactions.[8-10]

Many IN inhibitors are initially reported as potent inhibitors against HIV-1 IN-catalyzed 3′-processing (3′-P) and ST reactions in vitro. But these inhibitors failed to show good antiviral potencies in HIV-1 infected cells. Among the several IN inhibitors, the ′-diketo acidclass of compounds showed the most promising results. These class of compounds comprised of three important structural components (i.e.,) ′-diketo moiety, an aromatic or heteroaromatic portion and carboxylic acid group, which can be replaced with a variety of bioisosteric functions.[11,12] Among this class of compounds containing ′-diketo functional group, S-1360 [Figure 2 (1)] is the first IN inhibitor to enter into human clinical trials. S-1360 showed potent antiviral activity against a variety of HIV-1 clinical isolates; but unfortunately it failed to show efficacy in HIV-1 infected patients due to the metabolic instability.[13]

Raltegravir [Figure 2 (2)] developed by Merck, is the first IN inhibitor approved by the U.S. Food and Drug Administration (FDA) in 2007. It showed potent antiviral activity against a wide range of clinical HIV-1 isolates, including strains resistant to almost all clinically

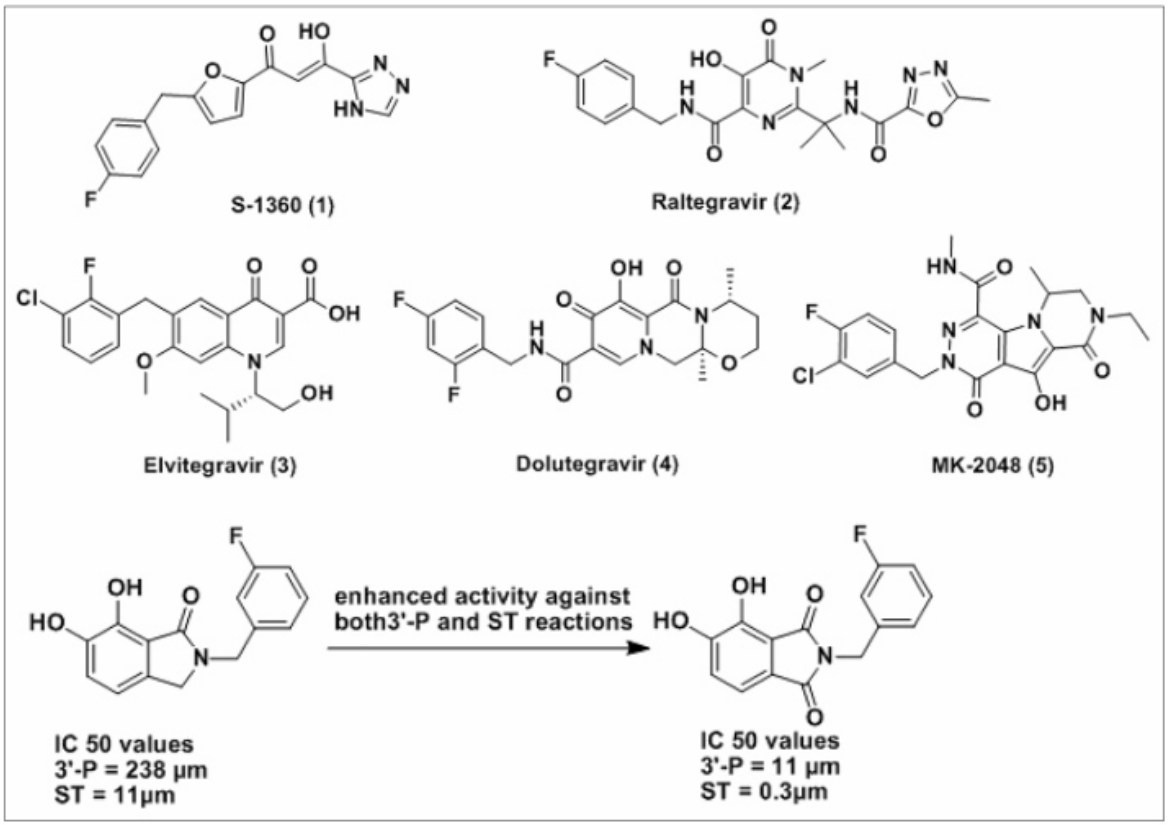


Figure 2: Structures of some integrase inhibitors

used antiretroviral drugs.[14] Elvitegravir [Figure 2 (3)] belongs to the quinolone-3-carboxylic acid class of IN inhibitors and approved by the U.S. FDA in 2012 to treat HIV patients.[15] Dolutegravir [Figure 2 (4)] is developed by GlaxoSmithKline, currently in late-stage clinical trials. Dolutegravir showing better results in the clinical trials.[16] MK-2048 [Figure 2 (5)] is the new second generation IN inhibitor developed by Merck in the year 2009, showing potent activity against Raltegravir and Elvitegravir resistance strains.[17]

Catechol based inhibitors are other important class of HIV-1 IN inhibitor. Increased potency was observed with maintained planar relationship between two hydroxyl groups on the aromatic ring. Zhao et al., reported anti-IN activity of 2,3-Dihydro-6,7-dihydroxy-1H-isoindol-1-one analogs with potent Ic50 values inhibiting both 3′-processing and ST reactions. Introduction of second carbonyl moiety on five membered lactam (isoindole-1-one to an isoindole-1,3-dione) showed enhanced activity against both 3′-processing (Ic50 value increased from 238 µm to 11 µm) and ST (Ic50 value increased from 11 µm to 0.3 µm) reactions.[16,18]

In light of these facts based upon an extensive perusal of literatures as well as our continued interest in the chemistry of N-substituted tetrahydrophthalimide, we have synthesized, characterized and evaluated HIV-1 IN inhibitory activity of novel 3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2

(3H,7H,7aH)-yl)-N-(substituted phenyl) propanamides.

MATERIALS AND METHODS

Chemistry

All solvents and chemicals purchased from Sigma or Merck companies were used as received without further purification. Solvent system used throughout the experimental work for running thin layer chromatography (TLC) was Ethyl acetate and Hexane (30:70) mixture to the monitor reaction.

Melting points are uncorrected and were determined in capillary tubes on a Precision Buchi B530 melting point apparatus containing silicon oil. IR spectra were recorded using a Jasco fourier transform infrared spectrophotometer (FT-IR). Proton nuclear magnetic resonance spectrophotometer 1H NMR spectra were recorded either on a Bruker DPX-400 spectrometer, using the TMS as an internal standard (chemical shifts in d ppm). The electron spray ionization mass spectras ESMS (m/z) were recorded on MICROMASS Quadro-II LCMS system.

Synthesis route for designed analogs 3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-(substituted phenyl) propanamide (4a-o) and intermediates 3-chloro-N-(substituted phenyl) propanamide analogs (2a-o) was outlined in Scheme 1. Both the intermediates and final compounds were prepared by following below given method.[19] Substitutions on aryl ring of the synthesized compounds, some physical data, and isolated yields are presented in Table 1.

EXPERIMENTAL

General procedure for synthesis of 3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-(substituted phenyl) propanamides 4

To a solution of 3a, 4,7,7a-tetrahydro-1H-isoindole-1,3 (2H)-dione (3) (2 mmol) in acetonitrile, potassium carbonate (6 mmol) and corresponding 3-chloro-N-(substituted phenyl) propanamides 2(a-o) (2 mmol) were added and refluxed for 8 h. On completion of the reaction as monitored by TLC, the contents were poured on crushed ice. Resulted precipitate was filtered, dried and recrystallized from ethanol to obtain pure product 4.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-phenylpropanamide (4a)

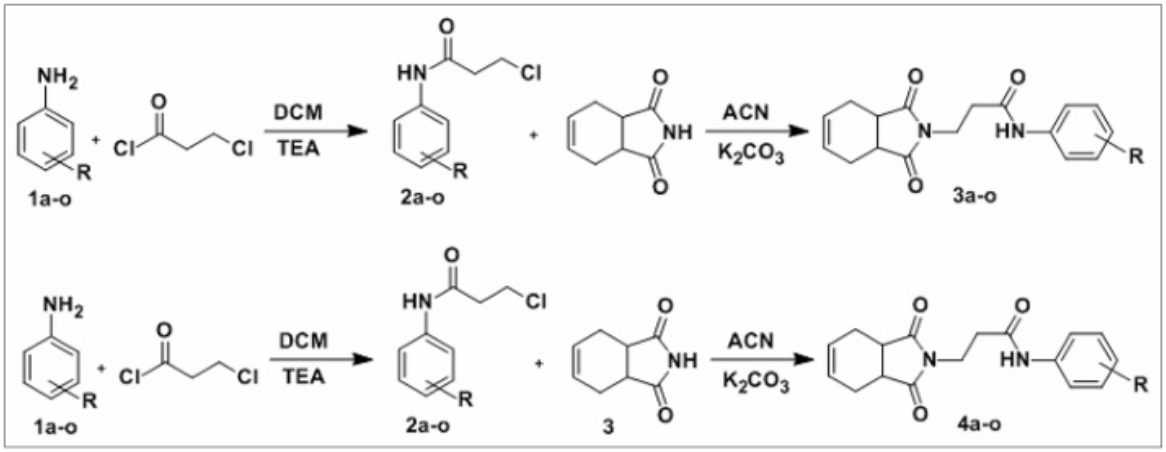
White solid, (80%, MP = 104-106°C). IR (KBr, cm-1): 3271 (N-H), 1776 and 1712 (C = O, isoindole), 1693 (C = O, amide), 1H NMR (CDCl3): d 2.17-2.64 (m, 2H, CHH, CHH), 2.57-2.64 (m, 2H, CHH, CHH), 2.68 (t, J = 8.0 Hz, 2H, NCH2), 3.07-3.13 (m, 2H, CH-CH), 3.87 (t, J = 8.0 Hz, 2H, COCH2), 5.78-5.85 (m, 2H, CH = CH), 7.10 (t, J = 8.0 Hz, 1H, ArH), 7.29 (dd, J = 16.0, 4.0 Hz, 2H, ArH), 7.50 (d, J = 8.0 Hz, 2H, ArH), 7.79 (brs, 1H, NH). MS (ES+): m/z = 299.6 [M + 1]. Elemental analysis: Calcd; C, 68.50; H, 6.25; N, 9.60.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-(4-methoxyphenyl) propanamide (4b)

White solid, (88%, MP = 106-108°C). IR (KBr, cm-1): 3305 (N-H), 1778 and 1710 (C = O, isoindole), 1697 (C = O, amide), 1249 (C-O-C). Elemental analysis: Calcd; C, 65.60; H, 6.30; N, 8.75. N-(4-chlorophenyl)-3-(1,3-dioxo-3a, 4-dihydro-1

H-isoindol-2 (3H,7H,7aH)-yl)propanamide (4c)

White solid, (78%, MP = 114-116°C). IR (KBr, cm-1): 3408 (N-H), 1772 and 1712 (C = O, isoindole), 1698 (C = O, amide), 689 (C-Cl). Elemental analysis: Calcd; C, 61.60; H, 5.25; N, 8.70.



Scheme 1: Protocol of synthesis

Table 1: Physical data and isolated yields of synthesized compounds

Comp. code	R	Mol. formula	Mol. weight	MP (°C)	Isolated %yield
4a	H	$C_{17}H_{18}N_2O_3$	298.34	104-106	80
4b	4-methoxy	$C_{18}H_{20}N_2O_4$	328.36	106-108	88
4c	4-chloro	$C_{17}H_{17}ClN_2O_3$	332.78	114-116	78
4d	4-methyl	$C_{18}H_{20}N_2O_3$	312.36	104-106	82
4e	3-methoxy	$C_{18}H_{20}N_2O_4$	328.36	82-84	72
4f	3-chloro	$C_{17}H_{17}ClN_2O_3$	332.78	102-104	70
4g	3-methyl	$C_{18}H_{20}N_2O_3$	312.36	96-98	76
4h	2-chloro	$C_{17}H_{17}ClN_2O_3$	332.78	108-110	70
4i	2-methyl	$C_{18}H_{20}N_2O_3$	312.36	100-102	72
4j	4-nitro	$C_{17}H_{17}N_3O_5$	343.33	142-143	72
4k	3-nitro	$C_{17}H_{17}N_3O_5$	343.33	100-102	68
4l	2-nitro	$C_{17}H_{17}N_3O_5$	343.33	104-106	64
4m	2,4 di methyl	$C_{19}H_{22}N_2O_3$	326.39	98-100	76
4n	3,4 di methyl	$C_{19}H_{22}N_2O_3$	326.39	110-112	78
4o	2,6 di methyl	$C_{19}H_{22}N_2O_3$	326.39	90-92	72

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-p-tolylpropanamide (4d)

White solid, (82%, MP = 104-106°C). IR (KBr, cm-1): 3363 (N-H), 1768 and 1706 (C = O, isoindole), 1698 (C = O, amide), Elemental analysis: Calcd; C, 69.35; H, 6.60; N, 8.80.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-(3-methoxyphenyl) propanamide (4e)

White solid, (72%, MP = 82-84°C). IR (KBr, cm-1): 3259 (N-H), 1774 and 1712 (C = O, isoindole), 1703 (C = O, amide), 1234(C-O-C). Elemental analysis: Calcd; C, 65.40; H, 6.00; N, 8.65. N-(3-chlorophenyl)-3-(1,3-dioxo-3a,

4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl) propanamide (4f)

White solid, (70%, MP = 102-104°C). IR (KBr, cm-1): 3342 (N-H), 1776 and 1712 (C = O, isoindole), 1682 (C = O, amide), 678 (C-Cl). Elemental analysis: Calcd; C, 61.20; H, 5.35; N, 8.30.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-m-tolylpropanamide (4g)

White solid, (76%, MP = 96-98°C). IR (KBr, cm-1): 3290 (N-H), 1768 and 1712 (C = O, isoindole), 1697 (C = O, amide). Elemental analysis: Calcd; C, 69.50; H, 6.20; N, 8.70.

N-(2-chlorophenyl)-3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl) propanamide (4h)

White solid, (70%, MP = 108-110°C). IR (KBr, cm-1): 3265 (N-H), 1772 and 1705 (C = O, isoindole), 1694 (C = O, amide), 697 (C-Cl). Elemental analysis: Calcd; C, 61.50; H, 5.05; N, 8.35.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl)-N-o-tolylpropanamide (4i)

White solid, (72%, MP = 100-102°C). IR (KBr, cm-1): 3302 (N-H), 1784 and 1702 (C = O, isoindole), 1676 (C = O, amide). Elemental analysis: Calcd; C, 69.15; H, 6.70; N, 8.95. 3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2

(3H,7H,7aH)-yl)-N-(4-nitrophenyl) propanamide (4j)

Yellow solid, (72%, MP = 142-143°C). IR (KBr, cm-1): 3325 (N-H), 1779 and 1710 (C = O, isoindole), 1686 (C = O, amide), 1542, 1322 (C-NO2). Elemental analysis: Calcd; C, 59.80; H, 4.80; N, 12.20.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2
(3H,7H,7aH)-yl)-N-(3-nitrophenyl) propanamide (4k)

Yellow solid, (68%, MP = 100-102°C). IR (KBr, cm-1): 3338 (N-H), 1774 and 1712 (C = O, isoindole), 1693 (C = O, amide), 1537, 1327 (C-NO2). E lemental analysis: Calcd; C, 59.50; H, 5.05; N, 12.35.

3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2
(3H,7H,7aH)-yl)-N-(2-nitrophenyl) propanamide (4l)

Yellow solid, (64%, MP = 104-106°C). IR (KBr, cm-1): 3331 (N-H), 1774 and 1714 (C = O, isoindole), 1698 (C = O, amide), 1531, 1336 (C-NO2). Elemental analysis: Calcd; C, 59.65; H, 4.80; N, 12.50.

N-(2,4-dimethylphenyl)-3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl) propanamide (4m)

White solid, (76%, MP = 99-100°C). IR (KBr, cm-1): 3284 (N-H), 1782 and 1712 (C = , isoindole), 1672 (C = O, amide). Elemental analysis: Calcd; C, 69.95; H, 6.90; N, 8.80.

N-(3,4-dimethylphenyl)-3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl) propanamide (4n)

White solid, Yield: 78%. MP 110-112°C, IR (KBr, cm-1): 3286 (N-H), 1778 and 1708 (C = O, isoindole), 1697 (C = O, amide), 1H NMR (CDCl3): d 2.20 (s, 3H, CH3), 2.23-2.24 (m, 2H, CHH, CHH), 2.28 (s, 3H, CH3), 2.59-2.62 (m, 2H, CHH, CHH), 2.67 (t, J = 8.0 Hz, 2H, NCH2), 3.09-3.10 (m, 2H, CH-CH), 3.88 (t, J = 8.0 Hz, 2H, COCH2), 5.84-5.86 (m, 2H, CH = CH), 6.98-7.00 (m, 2H, ArH), 7.11 (brs, 1H, NH), 7.51 (d, J = 8.0 Hz, 1H, ArH). MS (ES+): M/z = 327.4 [M + 1]. Elemental analysis: Calcd; C, 69.75; H, 6.60; N, 8.60.

N-(2,6-dimethylphenyl)-3-(1,3-dioxo-3a, 4-dihydro-1H-isoindol-2 (3H,7H,7aH)-yl) propanamide (4o)

White solid, (72%, MP = 90-92°C). IR (KBr, cm-1): 3253 (N-H), 1786 and 1714 (C = O, isoindole), 1695 (C = O, amide). Elemental analysis: Calcd; C, 69.80; H, 6.90; N, 8.75.

HIV-1 IN assay

IN was pre-incubated at a final concentration of 100 μM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonicacid 4-(2-hydroxyethyl)-1-piperazineethane sulfonicacid (HEPES), pH 7.5, 50 μM ethylene diamine tetraacetic acid ethylene diamine tetraacetic acid (EDTA), 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM MnCl2, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, and 25 mM 3(N-morpholino) propane sulfonicacid 3(N-morpholino) propane sulfonicacid (MOPS), pH 7.2) at 30°C for 30 min. Then, 20 nM of the 5′-end 32P-labeled linear oligonucleotide substrate was added, and the incubation was continued for an additional 1 h. Reactions were quenched by the addition of an equal volume (16 mL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, 0.025% bromophenol blue). An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea).

Gels were dried, exposed in a Molecular Dynamics Phosphorimager cassette, and analyzed using a Molecular Dynamics Phosphorimager (Sunnyvale, CA). Percent inhibition was calculated using the following equation:

$$I\% = 100 \times (1(D- C)/(N- C))$$

where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or ST products for DNA alone, DNA plus IN, and IN plus drug, respectively. Ic50 values were determined by plotting the drug concentration versus percent inhibition and determining the concentration, which produced 50% inhibition.[7]

RESULTS

All the designed analogs (4a-o) were synthesized by using the conditions mentioned in the Scheme 1. Synthesized compounds were characterized by FT-IR, 1H NMR, Mass spectral and Elemental Analysis data. They were tested for inhibitory activity against HIV-1 IN using 32P-labeled assay and results were shown in Figure 3.

[in Mn+2 containing reactions. Lane 1 and 18. DNA plus IN; lane 2 and 19. DNA alone; lanes 3 to17 DNA and

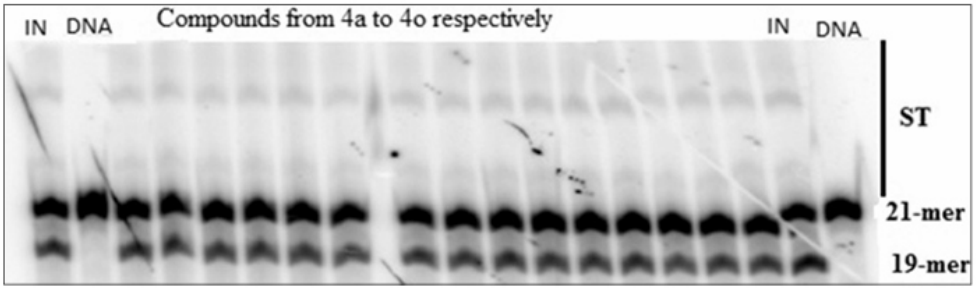


Figure 3: Concentration-dependent inhibition of HIV-1 IN by synthesized compounds 4a to 4o

IN in the presence of compounds 4a to 4o respectively (concentration of compounds is 100 µg/mL).

DISCUSSION

All the synthesized tetrahydrophthalimide analogs 4(a-o) were tested for HIV-1 IN inhibitory activity using 32P-labeled assays. Even though, all the above synthesized analogs contains 1,3-diketo functional groups in their structure, which is essential for anti-IN activity. In the study, none of the compounds showed any significant IN inhibitory activity (both 3′ processing and ST) at tested concentration 100 µg/mL. This may be due to a) substitution on 2nd position, i.e., in between two keto (1,3-diketo) functional groups. Hence because of some steric effects and electronic effects of the alkyl side chain, both keto groups may be unable to form chelating tripod with Mg2+ ion present in IN enzyme. b) The distance between carbonyl carbon (phthalimide) and amide carbonyl carbon present in the side chain is also more; hence they may be unable to form chelation with Mg2+ ion, which is essential for inhibition of HIV-1 IN functions.

CONCLUSION

In the present study, all the analogs were designed based on the essential pharmacophoric requirements for HIV-1 IN inhibitory activity. However, none of the synthesized compounds showed any significant anti HIV-1 IN activity. Hence, further detailed study will be required to find out the exact reason for lack of activity of the synthesized analogs as well as to generate analogs with significant HIV-1 IN inhibitory activity.

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REFERENCES

1. UNAIDS report on the global AIDS epidemic 2012. Available from: http://www.unaids.org/. [Up dated on 2012 Dec 1; cited 2012 Dec 15].

2. Chong P, Sebahar P, Youngman M, Garrido D, Zhang H, Stewart EL, et al. Rational design of potent non-nucleoside inhibitors of HIV-1 reverse transcriptase. J Med Chem 2012;55:10601-9.

3. Vandamme AM, Van Vaerenbergh K, De Clercq E. Anti-human immunodeficiency virus drug combination strategies. Antivir Chem Chemother 1998;9:187-203.

4. Chen IJ, Neamati N, Nicklaus MC, Orr A, Anderson L, Barchi JJ Jr, et al. Identification of HIV-1 integrase inhibitors via three-dimensional database searching using ASV and HIV-1 integrases as targets. Bioorg Med Chem 2000;8:2385-98.

5. Sippel M, Sotriffer CA. Molecular dynamics simulations of the HIV-1 integrase dimerization interface: Guidelines for the design of a novel class of integrase inhibitors. J Chem Inf Model 2010;50:604-14.

6. Gardelli C, Nizi E, Muraglia E, Crescenzi B, Ferrara M, Orvieto F, et al. Discovery and synthesis of HIV integrase inhibitors: Development of potent and orally bioavailable N-methyl pyrimidones. J Med Chem 2007;50:4953-75.

7. Dayam R, Al-Mawsawi LQ, Neamati N. Substituted 2-pyrrolinone inhibitors of HIV-1 integrase. Bioorg Med Chem Lett 2007;17:6155-9.

8. Dayam R, Al-Mawsawi LQ, Zawahir Z, Witvrouw M, Debyser Z, Neamati N. Quinolone 3-carboxylic acid pharmacophore: Design of second generation HIV-1 integrase inhibitors. J Med Chem 2008;51:1136-44.

9. Di Santo R, Costi R, Roux A, Miele G, Crucitti GC, Iacovo A, et al. Novel quinolinonyl diketo acid

derivatives as HIV-1 integrase inhibitors: Design, synthesis, and biological activities. J Med Chem 2008;51:4744-50.

10. Pasquini S, Mugnaini C, Tintori C, Botta M, Trejos A, Arvela RK, et al. Investigations on the 4-quinolone-3-carboxylic acid motif. 1. Synthesis and structure-activity relationship of a class of human immunodeficiency virus type 1 integrase inhibitors. J Med Chem 2008;51:5125-9.

11. Bacchi A, Biemmi M, Carcelli M, Carta F, Compari C, Fisicaro E, et al. From ligand to complexes. Part 2. Remarks on human immunodeficiency virus type 1 integrase inhibition by beta-diketo acid metal complexes. J Med Chem 2008;51:7253-64.

12. Sato M, Motomura T, Aramaki H, Matsuda T, Yamashita M, Ito Y, et al. Novel HIV-1 integrase inhibitors derived from quinolone antibiotics. J Med Chem 2006;49:1506-8.

13. Billich A. S-1360 Shionogi-GlaxoSmithKline. Curr Opin Investig Drugs 2003;4:206-9.

14. Boros EE, Edwards CE, Foster SA, Fuji M, Fujiwara T, Garvey EP, et al. Synthesis and antiviral activity of 7-benzyl-4-hydroxy-1,5-naphthyridin-2 (1H)-one HIV integrase inhibitors. J Med Chem 2009;52:2754-61.

15. Sax PE, DeJesus E, Mills A, Zolopa A, Cohen C, Wohl D, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: A randomised, double-blind, phase 3 trial, analysis of results after 48 weeks. Lancet 2012;379:2439-48.

16. Métifiot M, Maddali K, Johnson BC, Hare S, Smith SJ, Zhao XZ, et al. Activities, crystal structures, and molecular dynamics of dihydro-1H-isoindole derivatives, inhibitors of HIV-1 integrase. ACS Chem Biol 2013;8:209-17.

17. Pandey KK, Bera S, Vora AC, Grandgenett DP. Physical trapping of HIV-1 synaptic complex by different structural classes of integrase strand transfer inhibitors. Biochemistry 2010;49:8376-87.

18. Zhao XZ, Semenova EA, Vu BC, Maddali K, Marchand C, Hughes SH, et al. 2,3-dihydro-6,7-dihydroxy-1H-isoindol-1-one-based HIV-1 integrase inhibitors. J Med Chem 2008;51:251-9.

19. Ganguly S, Murugesan S, Maga G. Synthesis, evaluation and molecular modeling studies of some novel tetrahydroisoquinoline derivatives targeted at the HIV-1 Reverse Transcriptase. Indian J Heterocycl Chem 2009;18:357-60.

Incidence of new onset of type-2 diabetes with the use of atenolol for treatment of hypertension in north indian population: No role of irs-1 and kir 6.2 Gene polymorphism

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ABSTRACT

Introduction: *Previous research has suggested that* β1 *adreno-receptor blockers commonly used for management of hypertension may promote new onset of type-2 diabetes mellitus. The objective of this study is to evaluate the role of insulin receptor substrate-1 (IRS-1) gene and pancreatic ATP-sensitive potassium inward rectifying channel (Kir 6.2) genetic polymorphism in induction of diabetes mellitus with chronic use of* β1 ***blocker.*** *Materials and Methods: A total of 150 patients with essential hypertension aged between 17 and 65 years who were diagnosed with essential hypertension and prescribed atenolol therapy, were recruited. Of these, only 100 patients responding to atenolol were followed-up for 12 months for monitoring blood glucose level every month. The IRS-1 and pancreatic ATP-sensitive potassium channel Kir 6.2 (E23K) gene polymorphism were genotyped using genomic DNA extracted from the whole blood of the recruited patients by polymerase chain reaction and restriction fragment length polymorphism. Results: This study revealed that among the 100 patients responding to atenolol 27% showed a significant increase in the fasting blood sugar. Genotyping study of the recruited patients revealed a difference in allelic frequencies for IRS-1 (Gly972Arg) and pancreatic ATP-sensitive potassium channel Kir 6.2 (E23K) variants. However, allelic distribution between the hypertensive patients on atenolol showing hyperglycemia and normoglycemia was not significantly different for these genes. Conclusion: Thus, showing no correlation, for incidence of diabetes post atenolol therapy in the studied population with these gene polymorphisms.*

Key words: Atenolol, insulin receptor substrate-1, Kir 6.2 gene polymorphism, new onset of diabetes mellitus

INTRODUCTION:

During the past decade, several studies have shown that a large proportion of patients with hypertension are resistant to insulin stimulated glucose uptake.[1-4] Evidence of a relationship between insulin resistance and hypertension is increasing.[5] It is also becoming increasingly clear that antihypertensive medication have disparate effects on insulin sensitivity in patients with essential hypertension.[6] Some antihypertensives are associated with adverse metabolic effects including hyperglycemia, hypertriglyceridemia, and hyperuricemia.[7] Discussions regarding the use of antihypertensive agents and association of diabetes has focused on the negative metabolic effects of β-blockers.[8] Treatment with β-blockers increases insulin resistance,[9,10] thereby increasing the risk

of developing type 2 diabetes mellitus or impaired glucose tolerance.[11-15] Non-cardiac effects of atenolol point to a wider range of side-effects.[16]

Many candidate genes for type-2 diabetes have been proposed based on their role in insulin action or insulin resistance.[17,18] A meta-analysis of 32 case-controlled studies looking into association of insulin receptor substrate-1 (IRS-1) G972R polymorphism and type-2 diabetes proved to be inconclusive.[19] Since therapy with β-blockers has been shown to inhibit pancreatic insulin secretion, peripheral glucose utilization[20] and reduced insulin clearance[13] Therefore, it is hypothesized that antihypertensive-induced adverse metabolic effects, may be due to polymorphism of the IRS-1 and pancreatic ATP-sensitive potassium channel Kir 6.2 The gene encoding the IRS-1 protein has been localized to chromosome 2q35-q36.1 and has been studied extensively.[21] IRS-1 is a signaling protein that acts as a docking and activation site for multiple signaling molecules that control cellular growth and glucose metabolism.[22]

This gene encodes a protein, which is phosphorylated by insulin receptor tyrosine kinase. Mutations in this gene are associated with type 2 diabetes and susceptibility to insulin resistance. One of the most common mutations in the IRS-1 gene is at codon 972, where a point mutation G - A causes a change of glycine codon -GGG to arginine –AGG resulting in a non-synonymous amino acid change (Gly972Arg) at this position in the polypeptide chain.[23,24]

The ATP-sensitive potassium (KATP) channel is a key component regulating the release of insulin to maintain glucose homeostasis.[25,26] The KATP channel is a hetero-octameric protein complex comprised of the pore-forming inward-rectifier Kir 6.2 subunit coupled to the high-affinity sulfonylurea receptor subunit.[27,28] E23K polymorphism in KCNJ11 has been most extensively studied in classical form of type 2 diabetes. E23K is a missense single nucleotide polymorphism (SNP) (GAG → AAG) located in the cytosolic proximal (5') N- terminal of the Kir 6.2 subunit and results in the substitution of glutamate (E) with lysine (K).[29] Therefore, this study was undertaken to assess the correlation of adverse metabolic effects if any, of atenolol among the hypertensive patients from North Western India; with IRS-1 (rs 1801278; Gly972Arg) and pancreatic ATP-sensitive potassium channel Kir 6.2 (rs 5219; E23K) gene polymorphisms.

MATERIALS AND METHODS

Subjects Males or females (N = 150; 86 male, 64 female) with mild to moderate essential hypertension, of Asian Indian ethnicity residing at Sriganganagar, Rajasthan, North Western India were being recruited to participate in this study as per the following criteria:

Inclusion criteria

- Age 17-65 years

- Average home diastolic blood pressure (DBP) >85 mm Hg and office DBP >90 mm Hg.

Exclusion criteria

- Office or average home DBP >110 mm Hg
- Office or average home systolic blood pressure >180 mm Hg
- Secondary forms of hypertension (including sleep apnea)
- Diabetes mellitus (type 1 or 2) or screening fasting blood glucose >120 mg/dL
- Pregnancy or lactation
- Chronic treatment with blood pressure (BP)-elevating drugs (including nonsteroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, and oral contraceptives)
- Drug or alcohol use likely to affect study protocol adherence.

Protocol

The study protocol was approved by the Institutional Ethics Committee for human participants of Seth G.L. Bihani S.D. College technical education Sri Ganganagar, Rajasthan vide No. 1/19-02-2008. The subjects were followed-up for 12 months for monitoring of fasting blood glucose every month. Criteria for development of glucose metabolic dysfunction were fasting blood sugar (FBS) above 120 mg/dL.

Anthropometric measurements

Height and weight were measured to the nearest 0.1 cm and 0.5 kg, respectively. Body mass index (BMI) was calculated with the formula: Weight (kg)/height (m2).

Laboratory measurements

Blood glucose monitoring was done by glucose oxidase-peroxidase, end point assay method using Span Diagnostic Kits, Gujarat.

Determination of genotypes

All the responders of antihypertensive medication were genotyped for IRS-1 and E23K gene polymorphism. The blood samples (5 ml) were collected in the ethylenediaminetetraacetic acid (EDTA) coated tubes and processed for isolation of DNA. DNA was extracted with a DNA extraction kit from (Bengaluru Genei, Bengaluru) as described in the manufacturer's protocol. The quantified DNA was diluted to final concentration of 25 ng/µl in Tris-EDTA buffer (10 mM Tris Cl, 1 mM EDTA, pH 8.0).

Genotyping for insulin receptor substrate-1 gene polymorphism

DNA samples of study subjects were genotyped for Gly972Arg polymorphism of IRS-1 gene using forward primer 5'- GCAGCCTGGCAGGAGAGCACT- 3' and reverse primer 5'- CTCACCTCCTCTGCAGCAATG - 3'. Polymerase chain reaction (PCR) reactions were performed in final volume of 25 µl containing ×10 assay buffer (Bangalore Genei), 0.5 units of Taq DNA polymerase (Bangalore Genei), 200 µmole of each deoxynucleotide triphosphates (dNTP's) (Bangalore Genei), 10 pmole/reaction of each forward and reverse primers and 50 ng of template DNA. Initial denaturation for 6 min at 94°C, followed by 35 cycles for denaturation for 1 min at 94°C, primer annealing for 1 min at 61.3°C and extension for 30 s at 72°C. The amplified DNA fragments were digested using 10 µl of PCR product with 3U of BstN I (CC/WGG). The digestion mixture was incubated at 56°C for 1 h.[30]

Genotyping for Kir 6.2 gene polymorphism

DNA samples of study subjects were genotyped for E23K polymorphism using forward Primer 5'-CAGTTGCCTTTCTTGGACACAAA-3' and Reverse 5'-CCGAGGAATACGTGCTGACA-3'. PCR reactions were performed in final volume of 25 µl containing ×10 assay buffer (Bangalore Genei), 0.5 units of Taq DNA polymerase (Bangalore Genei), 200 µmole of each dNTP's (Bangalore Genei), 10 pmole/reaction of each forward and reverse primers and 50 ng of template DNA. [31] Initial denaturation for 6 min at 94°C, 35 amplification cycles were performed with denaturation for 1 min at 94°C, primer annealing for 1 min at 67°C and extension for 30 s at 72°C. The amplified DNA fragments were digested using 10 µl of PCR product with 3U of Ban II (GRGCY/C).[31]

Finally, the digested PCR products were analyzed on a 1% agarose gel after an electrophoresis at a constant voltage of 100 V for 90 min.

Genotype analysis

Amplification of IRS-1 yielded a product of 220 bp. Digestion of IRS-1 gene amplification product yielded two fragments of 164 bp and 56 bp in the presence of the variant "A" allele [Figure 1]. The wild type allele "G" was not digested by BstN1. Thus, homozygous GG showed only one band, homozygous AA yielded two fragments of 164 bp and 56 bp and heterozygous GA all three bands.

In case of Kir 6.2 gene polymorphism a 218 bp fragment containing the SNP site was amplified. Digestion of

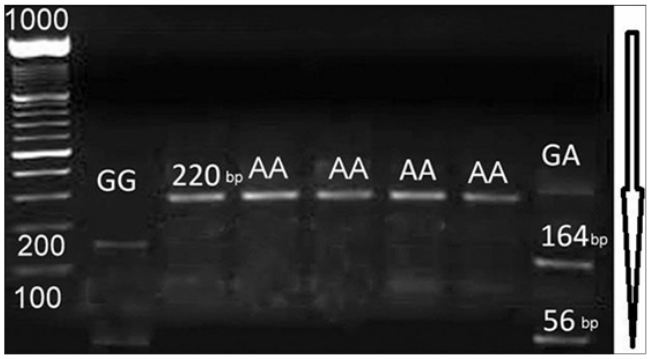

Figure 1: Agarose gel photograph for Insulin Receptor Substrate 1 gene polymorphism

IRS-1 gene product, in presence of wild type allele E, that is, GAG; yielded two fragments of 178 bp and 40 bp. The product was not digested in homozygous variant polymorphic allele. Heterozygous genotype showed both digested and intact gene products [Figure 2].

Statistical analysis

Nonparametric tests were used as the data were not normally distributed. Baseline characteristics were compared between normo-glycemic and hyperglycemic patients using Chi-square test. Allelic distribution was analyzed using Hardy-Weinberg calculator. All the statistical analyses were performed using SPSS 17 (IBM, USA).

RESULTS

A total of 150 patients (86 male, and 64 female) with primary hypertension were recruited. The anthropometric measurements of these patients were recorded [Table 1] before the study. Of the 150 patients recruited for the study initially 50 were found to be non-responders, hence were not analyzed any further. Of the 100 responders who continued on atenolol therapy, 27 patients were found associated with significant increase in FBS when they were compared with another 73 responders ($P < 0.0001$).

Among these, 27 patients who showed increase in FBS 17 were male and 10 were female. Average onset time was found to be 5-6 months. Table 2 summarizes comparison of mean age, BMI, FBS, gender-wise differences among the patients with and without metabolic dysfunction after 12 months of treatment.

Genotyping study of the responder patients did not reveal any significant difference in allelic frequencies for IRS-1 (Gly972Arg) and pancreatic ATP-sensitive potassium channel Kir 6.2 (E23K) variants among the two sexes, thus the data were pooled for further analysis [Tables 3 and 4].

Further a Comparison of genotypes and allelic distribution of IRS-1 gene and Kir 6.2 gene in atenolol treated hypertensive patients with respect to age, BMI, systolic and diastolic BP and FBS before and after treatment revealed no significant difference [Tables 5-8].

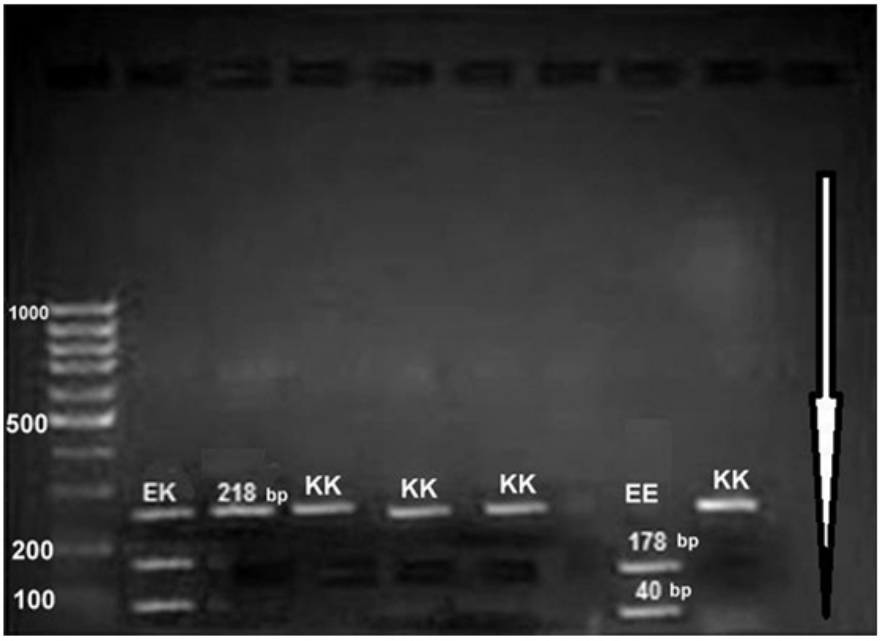

Figure 2: Agarose gel photograph for Kir 6.2 gene polymorphism

Table 1: The baseline anthropometric and clinical characteristics of recruited patients

Anthropometric and clinical characteristics of study subjects	Observed values
Total number of patients	150
Male	86
Female	64
Age (years)	55.073±0.921
BMI (kg/m^2)	24.64±0.416
SBP (mm of Hg±SD)	140.59±1.26
DBP (mm of Hg±SD)	90.6±0.55
FBS 1 (mg/dL±SD)	88.95±0.3470

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; SD: Standard deviation; FBS: Fasting blood glucose

Table 2: Comparison of different anthropometric and clinical characteristics between postatenolol treated hypertensive patients with and without metabolic dysfunction n number of patients

Anthropometric and clinical characteristics of study subjects	Without metabolic dysfunction	With metabolic dysfunction	*P* value
n	73 (52 male+ 21 female)	27 (17 male+ 10 female)	-
Age (years)	53.12±1.53	52.74±1.92	0.12
BMI	24.6±0.64	24.18±0.58	0.372
SBP	121.6±1.93	123.77±3.16	0.76
DBP	85.99±0.821	83.22±1.55	0.207
FBS 1	88.93±0.50	89.70±0.88	0.74
FBS 12	105.24±1.40	137.48±0.34	0.0001

BMI: Body mass index (kg/m²); FBS 1, 12: Fasting blood sugar at the start of study; at the end of 12 months respectively (mg/dL); SBP: Systolic blood pressure (mm of Hg); DBP: Diastolic blood pressure (mm of Hg)

DISCUSSION

In this study, 27 responders of a total of 100 responders of atenolol therapy showed a significant increase in FBS and were classified as having developed metabolic dysfunction after atenolol treatment. Among the causation of metabolic dysfunction, β1 blocker have been reported to induce disturbance in glucose metabolism resulting in weight gain.[18] However in this study, the risk of new onset of type-2 diabetes mellitus with atenolol therapy was found to be independent of age, BMI and sex of patients. Further role of genetic variations was investigated. Many candidate genes for type-2 diabetes have been proposed based on their role in insulin action or insulin resistance.[17,18,32,33] As, it was first study in Indian population to study genetic basis of this problem, a prospective study was planned to delineate the role of polymorphism of two key candidate genes IRS-1 and pancreatic ATP-sensitive potassium channel Kir 6.2 in the hypertensive patients responding to atenolol for control of hypertension.

One of the most commonly studied SNP rs 1801278 in the IRS-1 gene at codon 972, causes a missense change from glycine (GGG) to arginine (AGG).[23,24] resulting in three possible genotypes (Gly/Gly, Arg/Arg, Gly/Arg). This SNP is located within a tyrosine phosphorylation motif in the IRS-1 gene thus having functional significance for post translational modification of the protein. No

Table 3: Allelic distribution of IRS-1 G972A polymorphism among responders to atenolol

Genotype	GG	GA	AA	Allele frequency	χ^2 S/NS
Male	47	00	22	G=0.681 A=0.319	5.33 df=2
Female	17	02	12	G=0.581 A=0.419	*P*=0.069 NS
Total	64	02	34	G=0.650 A=0.350	

IRS: Insulin receptor substrate 1; NS: Nonsignificant

Table 4: Allelic distribution of Kir 6.2 E23K polymorphism among responders to atenolol

Genotype	EE	EK	KK	Allele frequency	χ^2 S/NS
Male	53	00	16	E=0.768 K=0.232	2.37 df=2
Female	24	01	06	E=0.790 K=0.209	*P*<0.30 NS
Total	77	01	22	E=0.775 K=0.225	

NS: Nonsignificant

Table 5: Comparison of different anthropometricand clinical parameters between atenolol treated hypertensive patients with and without metabolic dysfunction amongst different genotypes of IRS1 G927A alleles

Genotype	Without metabolic dysfunction 73 (52 male+21 female)			*P* value	With metabolic dysfunction 27 (17 male+10 female)			*P* value
	GG	AA	GA		GG	AA	GA	
Age (years)	51.23±2.08	55.54±2.23	52.0±2.0		52.68±2.29	52.88±3.82	-	0.96^{ns}
n	45	26	02		19	8		
BMI	24.10±0.51	25.77±1.56	23.22±0.99	0.44^{ns}	23.96±0.75	24.71±0.87	-	0.56^{ns}
SBP (before treatment)	140.3±2.30	142.9±3.46	155.0±25	0.42^{ns}	147.4±3.96	135.3±3.85	-	0.07^{ns}
SBP (after treatment)	128.7±2.04	129.8±3.44	139.5±22.50	0.62^{ns}	133.4±3.58	123.2±2.67	-	0.09^{ns}
DBP (before treatment)	90.66±1.07	91.46±1.37	92.50±7.50	0.86^{ns}	94.32±1.87	90.63±2.73	-	0.28^{ns}
DBP (after treatment)	83.20±0.96	83.05±1.36	83.25±6.75	0.99^{ns}	85.42±1.83	82.56±1.94	-	0.36^{ns}
FBS 1	88.52±0.64	89.42±0.91	90.0±0.0	0.66^{ns}	89.58±1.06	90.0±0.0	-	0.86^{ns}
FBS 12	106.3±2.75	104.9±3.14	92.50±2.50	0.53^{ns}	137.7±0.97	137.0±0.34	-	0.70^{ns}

BMI: Body mass index (kg/m2); FBS 1, 12: Fasting blood sugar at the start of study; at the end of 12 months respectively (mg/dL); SBP: Systolic blood pressure (mm of Hg); DBP: Diastolic blood pressure (mm of Hg); IRS: Insulin receptor substrate 1; ^{ns}Nonsignificant

Table 6: Comparison of different clinical parameters between atenolol treated hypertensive patients with and without metabolic dysfunction amongst different genotypes of Kir 6.2 E23K alleles

Genotype	Without metabolic dysfunction 73 (52 male+21 female)			*P* value	With metabolic dysfunction 27 (17 male+10 female)			*P* value
	EE	KK	EK		EE	KK	EK	
Age (years)	52.46±1.79	56.71±2.88	47.0±11.0	0.44^{ns}	51.95±2.47	55.0±2.41	-	0.49^{ns}
BMI	24.96±0.79	23.45±0.74	22.63±4.03	0.58^{ns}	23.60±0.55	25.83±1.50	-	0.09^{ns}
SBP (before treatment)	140.1±2.08	144.3±4.62	165.0±15	0.08^{ns}	142.6±3.69	147.1±6.44	-	0.53^{ns}
SBP (after treatment)	128.1±1.97	131.9±4.17	148.5±13.5	0.14^{ns}	129.3±3.21	133.4±5.69	-	0.52^{ns}
DBP (before treatment)	90.02±0.90	93.64±1.83	100±0.0	0.03*	94.50±1.94	89.57±1.74	-	0.16^{ns}
Diastolic BP (after treatment)	82.27±0.85	85.58±1.65	89.50±0.50	0.08^{ns}	85.73±1.75	81.271.83	-	0.17^{ns}
FBS 1	88.75±0.58	89.50±1.10	90±0.0	0.79^{ns}	90.10±0.49	88.57±2.60	-	0.38^{ns}
FBS 12	105.7±2.42	104.4±3.64	97.50±2.50	0.78^{ns}	137.6±0.98	137.1±1.38	-	0.80^{ns}

BMI: Body mass index (kg/m^2); FBS 1, 12: Fasting blood sugar at the start of study; at the end of 12 months respectively (mg/dL); SBP: Systolic blood pressure (mm of Hg); DBP: Diastolic blood pressure (mm of Hg); ^{ns}Nonsignificant * significant

Table 7: Comparative allelic distribution of IRS-1 genes among post atenolol treated hypertensive patients with and without metabolic dysfunction

Group of patients (%)	IRS-1 gene genotype			Allele frequencies	Chi-square/ df/*P*/S/NS
	GG	AA	GA		
Without metabolic dysfunction	45	26	02	G=0.79 A=0.21	1.182 df=2
With metabolic dysfunction	19	8	00	G=0.85 A=0.15	*P*=0.55 NS

IRS-1: Insulin receptor substrate 1; NS: Nonsignificant

Table 8: Comparative allelic distribution of Kir 6.2 genes among post atenolol treated hypertensive patients with and without metabolic dysfunction

Group of Patients (%)	Kir 6.2 gene genotype			Allele frequencies	Chi-square/ df/*P*/S/NS
	EE	EK	KK		
Without metabolic dysfunction	57	02	14	E=0.88 K=0.12	1.20 df=2
With metabolic dysfunction	20	00	07	E=0.74 K=0.26	*P*=0.54 NS

NS: Nonsignificant

Gly/Arg heterozygous patient was observed in the group with elevated FBS. However, it was observed that patients with Gly as residue 972 as in case of genotypes Gly/Gly and Arg/Arg; a significant increase in FBS was recorded [Table 5]. A further perusal of allele distribution amongst such patients did not reveal a significant difference between patients with and without elevated FBS [Table 7]. Thus keeping the question of phramacogenetic effect unresolved.

The second gene Kir 6.2 was studied for rs 5219 a missense SNP (GAG → AAG) located in the cytosolic proximal (5') N- terminal of the Kir 6.2 subunit that results in the substitution of glutamate (E) with lysine (K) at position 23 in the amino acid chain.[29] The three possible genotypes (E/E, E/K,

K/K) were observed among the population of Sriganganagar, Rajasthan. It was found that both homozygous E/E and K/K variants shows a significant increase in FBS but no heterozygote E/K genotype was observed among this group [Table 6]. As no significant difference was observed between hyperglycemic and normoglycemic subjects after treatment with atenolol [Table 8] with respect to distribution of polymorphic alleles for rs 5219, it does not provide conclusive evidence for any correlation.

CONCLUSION

This study revealed significant risk for the onset of metabolic dysfunction in the form of elevated FBS in hypertensive patients treated with atenolol, a beta-adrenergic blocking agent within 5-6 months of treatment. However, the SNPs of IRS-1 (Gly972Arg) and pancreatic ATP-sensitive potassium channel Kir 6.2 (E23K) genes did not show any correlation with incidence of hyperglycemia due to antihypertensive therapy with atenolol in this population. However, it needs to be further investigated with other genes from the beta-adrenergic pathway, so that hypertensive patients can be stratified based on their genotype for safe therapy with atenolol.

REFERENCES

1. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, et al. Insulin resistance in essential hypertension. N Engl J Med 1987;317:350-7.

2. Pollare T, Lithell H, Selinus I, Berne C. Application of prazosin is associated with an increase of insulin sensitivity in obese patients with hypertension. Diabetologia 1988;31:415-20.

3. Pollare T, Lithell H, Berne C. Insulin resistance is a characteristic feature of primary hypertension independent of obesity. Metabolism 1990;39:167-74.

4. Reaven GM. Relationship between insulin resistance and hypertension. Diabetes Care 1991;14 Suppl 4:33-8.

5. Sowers JR. Insulin resistance and hypertension. Am J Physiol Heart Circ Physiol 2004;286:H1597-602.

6. Perez-Stable E, Caralis PV. Thiazide-induced disturbances in carbohydrate, lipid, and potassium metabolism. Am Heart J 1983;106:245-51.

7. Lithell HO. Effect of antihypertensive drugs on insulin, glucose, and lipid metabolism. Diabetes Care 1991;14:203-9.

8. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.

9. Pollare T, Lithell H, Selinus I, Berne C. Sensitivity to insulin during treatment with atenolol and

metoprolol: A randomised, double blind study of effects on carbohydrate and lipoprotein metabolism in hypertensive patients. BMJ 1989;298:1152-7.

10. Pollare T, Lithell H, Berne C. A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. N Engl J Med 1989;321:868-73.

11. Lundgren H, Björkman L, Keiding P, Lundmark S, Bengtsson C. Diabetes in patients with hypertension receiving pharmacological treatment. BMJ 1988;297:1512.

12. Skarfors ET, Selinus KI, Lithell HO. Risk factors for developing non-insulin dependent diabetes: A 10 year follow up of men in Uppsala. BMJ 1991;303:755-60.

13. Sowers JR. Is hypertension an insulin-resistant state? Metabolic changes associated with hypertension and antihypertensive therapy. Am Heart J 1991;122:932-5.

14. Mykkänen L, Kuusisto J, Pyörälä K, Laakso M, Haffner SM. Increased risk of non-insulin-dependent diabetes mellitus in elderly hypertensive subjects. J Hypertens 1994;12:1425-32.

15. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. N Engl J Med 2000;342:905-12.

16. Chopra HK, Krishna CK, Ravinder SS, Komal KK. Non-cardiac effects of atenolol. Suppl J Assoc Physicians India 2009;57:26-8.

17. Moller DE, Bjørbaek C, Vidal-Puig A. Candidate genes for insulin resistance. Diabetes Care 1996;19:396-400.

18. Sacks DB, McDonald JM. The pathogenesis of type II diabetes mellitus. A polygenic disease. Am J Clin Pathol 1996;105:149-56.

19. Morini E, Prudente S, Succurro E, Chandalia M, Zhang YY, Mammarella S, et al. IRS1 G972R polymorphism and type 2 diabetes: A paradigm for the difficult ascertainment of the contribution to disease susceptibility of 'low-frequency-low-risk' variants. Diabetologia 2009;52:1852-7.

20. Sawicki PT, Siebenhofer A. Beta-blockers and diabetes mellitus. J Clin Basic Cardiol 2001;4:17-20.

21. Panz VR, Raal FJ, O'Rahilly S, Kedda MA, Joffe BI. Insulin receptor substrate-1 gene variants in lipoatrophic diabetes mellitus and non-insulin-dependent diabetes mellitus: A study of South African black and white subjects. Hum Genet 1997;101:118-9.

22. Myers MG Jr, White MF. The new elements of insulin signaling. Insulin receptor substrate-1 and proteins with SH2 domains. Diabetes 1993;42:643-50.

23. Imai Y, Fusco A, Suzuki Y, Lesniak MA, D'Alfonso R, Sesti G, et al. Variant sequences of insulin receptor substrate-1 in patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1994;79:1655-8.

24. Almind K, Bjørbaek C, Vestergaard H, Hansen T, Echwald S, Pedersen O. Aminoacid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. Lancet 1993;342:828-32.

25. Ashcroft FM, Rorsman P. Electrophysiology of the pancreatic beta-cell. Prog Biophys Mol Biol 1989;54:87-143.

26. Reimann F, Gribble FM. Glucose-sensing in glucagon-like peptide-1-secreting cells. Diabetes 2002;51:2757-63.

27. Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, et al. Reconstitution of IKATP: An inward rectifier subunit plus the sulfonylurea receptor. Science 1995;270:1166-70.

28. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, González G, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: A regulator of insulin secretion. Science 1995;268:423-6.

29. Riedel MJ, Steckley DC, Light PE. Current status of the E23K Kir6.2 polymorphism: Implications for type-2 diabetes. Hum Genet 2005;116:133-45.

30. Lei HH, Coresh J, Shuldiner AR, Boerwinkle E, Brancati FL. Variants of the insulin receptor substrate-1 and fatty acid binding protein 2 genes and the risk of type 2 diabetes, obesity, and hyperinsulinemia in African-Americans: The Atherosclerosis Risk in Communities Study. Diabetes 1999;48:1868-72.

31. Shaat N, Ekelund M, Lernmark A, Ivarsson S, Almgren P, Berntorp K, et al. Association of the E23K polymorphism in the KCNJ11 gene with gestational diabetes mellitus. Diabetologia 2005;48:2544-51.

32. Seino S. Recent progress in the molecular genetic aspects of non-insulin-dependent diabetes mellitus. Intern Med 1996;35:347-55.

33. Groop LC. The molecular genetics of non-insulin-dependent diabetes mellitus. J Intern Med 1997;241:95-101.

Insignificant antidermatophytic activity of Brassica campestris oil

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ABSTRACT

Introduction: *The aim of the present study was to investigate the antidermatophytic activity of Brassica campestris oil against selected dermatophytes through a disc diffusion technique.*
Materials and Methods: *Four concentrations of mustard oil, 100%, 75%, 50%, and 25%, were screened against Trichophyton rubrum, Trichophyton simii, Chrysosporium indicum, and Chrysosporium tropicum through the disc diffusion technique.*
Results: *The result showed that 25% and 50% concentrations of oil did not show any zone of inhibition. 75% and 100% concentration showed very poor activity against T. rubrum, T. simii, and C. indicum but in the case of C.tropicum, no zone of inhibition was observed. Conclusion: The mustard oil does not exhibited significant antidermatophytic activity in the disc diffusion method.*

Key words: Dermatophytes, dermatophytosis, fungi, griseofulvin, trichophyton

INTRODUCTION

Dermatophytoses pose a serious concern to the sociologically backward and economically poor population of India.[1-3] Dermatophytoses represents systemic or deep fungal infections that may have prominent cutaneous and systemic manifestations. The disease is predominant in tropical and sub-tropical countries due to their prevailing moisture and temperature regimes and pose a therapeutic problem. Despite the availability of new systemic antifungal therapies, dermatophytic infections are difficult to eradicate completely, with recurrence reported in up to 25-40% of cases.[4] Many antifungal synthetic drugs namely imidazoles, butanafine, and terbinafine are effective in the treatment of dermatophytoses[5] but disease recurrence, resistant dermatophytic strains, and adverse effects are some drawbacks associated with popular antifungals.[6] In the present scenario, plants and their products have gained more importance as a possible source of alternative and effective drugs. Because of the long history of plants in the treatment of different human ailments, most of the herbal drugs are believed to be safer than the synthetic drugs with no side effects. Plants remain as an untapped reservoir of potentially useful chemical compounds not only as drugs but also as unique templates that could serve as a starting point for synthetic analogs.[7-10]

Brassica campestris belonging to the family Brassicaceae is commonly known as mustard. Mustard oil

has about 60% monounsaturated fatty acids (42% erucic acid and 12% oleic acid); it has about 21% polyunsaturated fats (6% the omega-3 alpha-linolenic acid and 15% the omega-6 linoleic acid) and it has about 12% saturated fats.

In our previous studies, flower, leaves, and stem parts of B. campestris plant were extracted for their water, methanol, free and bound extracts against dermatophytes and found excellent results.[11] These finding prompted us to explore other plant products that could be exploited as antifungal. Antimicrobial activity of mustard oil has been studied by various workers.[12,13] Therefore, in the present investigation, we used B. campestris oil against selected dermatophytes.

MATERIALS AND METHODS

Mustard oil was procured from the authorized Engine marked company store from Jaipur. The oil was store in amber color bottle in a refrigerator.

Micro organism for in vitro studies

B. campestris oil was evaluated for their antifungal properties against selected pathogens. T. rubrum and T. simii were isolated from infected skin scrapings of Tinea patients from SMS Hospital, Jaipur, while C. tropicum and C. indicum were isolated from soil samples through To.Ka.Va. hair-baiting technique of Vanbreuseghem.[14] These fungi were maintained on Sabouroud's dextrose agar medium.

Screening of Oil

The filter paper disc diffusion assay by Wannisorn et al.[15] was used with slightly modification for screening the essential oils against dermatophytes. Standard size whatman no. 1 filter paper discs 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for 1 hour were used to determine antifungal activity. 20 ml sterilized Sabouraud's dextrose agar medium was taken in each autoclaved Petri dish and allowed to solidify. Fungal spore suspension was prepared in sterilized 0.85% saline water by transferring a loopful of 15 day-old culture. 1 ml of spore suspension of approximately 0.5 to 5 × 104 (cfu/ml) was spread over the respective agar medium plates. Sterilized filter paper were soaked in neat undiluted oil. An oil saturated disc was placed on an agar plate containing fungal spore suspension. Ketoconazole was used as a standard drug. These plates were incubated. Five replicates were kept in each case and the average values were determined and inhibition zones were observed. The antifungal activity was determined by measuring the inhibition zone around the disc. The activity of oil was measured by the following formula.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition Zone (IZ) of samples}}{\text{Inhibition Zone (IZ) of standard}}$$

RESULTS AND DISCUSSION

During the present investigation, the disc diffusion method was not found to be good for the screening of mustard oil against test dermatophytes. All the four concentrations of mustard oil could not exhibit good antifungal properties against these test fungi. According to data incorporated in Table 1, Chrysosporium tropicum was found to be a resistant strain with all the four concentrations of mustard oil. Seventy-five percent and 100% concentration of oil showed little activity against Trichophyton rubrum, T. simii, and C. indicum. The maximum zone of 10 mm was observed when 100% concentrated oil was used against T. rubrum and T. simii. However, the maximum AI = 0.529 was seen against C. indicum. Fifty percent and 25% oil did not exhibit any response against these fungi. When the activity of oil was compared with standard drug, Griseofulvin, Itraconazole, and Ketoconazole, it was found that mustard oil is a very poor agent against selected fungi in the present study. In our previous work,[11] free and bound flavonoid fractions of leaf, flower, and pod of B. campestris showed the excellent antidermatophytic activity as compared to standards. Previous reports[12,13,16] showed B. campestris oil as effective antifungal but present studies showed negative result. In the present investigation, we used the disc diffusion method. Mustard oil is very viscous oil which could not be diffused as compared to other essential oil. However, in other method like the food poisoning method, we add oil in liquid medium containing fungal inoculum where oil show effective result. The present investigation concluded that disc diffusion technique is not an effective technique for viscous oil like mustard oil.

Table 1: Comparison of efficacy of Brassica campestris oil with commercial antifungal drugs

Concentrations of Oil (%)	Test Fungi													
	Trichophyton rubrum				Trichophyton simii				Chrysosporium indicum		Chrysosporium tropicum			
	IZ	AI			IZ	AI			IZ	AI	IZ	AI		
		TC/G	TC/I	TC/K		TC/G	TC/I	TC/K		TC/K		TC/G	TC/I	TC/K
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	8	0.286	0.381	0.157	9	0.375	0.45	0.243	7	0.412	-	-	-	-
100	10	0.357	0.476	0.196	10	0.417	0.5	0.270	9	0.529	-	-	-	-

IZ: Inhibition zone including 6 mm diameter of filter paper disc; AI: Activity index; TC: Test compound. Inhibition zones of standard Griseofulvin (G) against T. rubrum=28 mm; T. simii=24 mm; C. tropicum=35 mm. Inhibition zones of standard Itraconazole (I) against T. rubrum=21 mm; T. simii=20 mm; C. tropicum=17 mm. Inhibition zones of standard Ketoconazole (K) against T. rubrum=51 mm; T. simii=37 mm; C. tropicum=39 mm; C. indicum=17 mm.

RERERENCES

1. Bhadauria S, Jain N, Sahrma M, Kumar P. Dermatophytosis in Jaipur: Study of incidence, clinical feature and causal agent. Indian J Microbiol 2001;41:207-10.

2. Bindu V, Pavithran K. Clinicomycological study of dermatophytes in Calicut. Indian J Dermatol Venerol Leprol 2002;68:259-61.

3. Kannan P, Janaki C, Selvi GS. Prevlence of dermatophytes and other fungal agents isolated from clinical samples. Indian J Med Microbiol 2006;24:212-5.

4. Hay RJ. The future of onychomycosis therapy may involve a combination of approaches. Br J Dermatol 2001;145:3-8.

5. Jacob Z, Wahab S, Ghosh M, Shrivastava OP. Superficial mycoses and in vitro sensitivity of dermatophytes and Candida species to tolciclate and clotrimazole. Indian J Med Res 1981;74:365-71.

6. Artis WM, Odle BM, Jones HE. Griseofulvin resistant dermatophytosis correlates with in vitro resistance. Arch Dermatol 1981;117:16-9.

7. Jain N, Sharma M. Broad spectrum antimycotic drug for the treatment of ring worm infection in human beings. Curr Sci 2003;85:30-4.

8. Bhadauria S, Kumar P. Broad spectrum antidermatophytic drug for the control of tinea infection in human beings. Mycoses 2012;55: 339-43.

9. Lima EO, Gompertz OF, Giesbrecht AM, MQ. Paulo. In vitro antifungal activity of essential oil obtained from officinal plants against dermatophytes. 1993;36:333-6.

10. Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethanopharmacol 2001;74:217-20.

11. Jain N, Sharma M, Kumar P. Regulatory effect of some plant extract on the growth of dermatophytic fungi. Indian J Microbiol 2004;44:59-64.

12. Prasad RY, Alankararao GS, Baby P. Antimicrobial studies on the seed oil of Brassica juncea. Fitoterpia 1993;64:373-4.

13. Meena MR, Sethi V. Antimicrobial activity of essential oil from spices. J Food Sci Tech 1994;31:68-70.

14. Vanbreuseghem R. Technique biologique pour! isolements des dermatophyte du soil (Biological technique for the isolation of dermatophytes from the soil). Ann Soc Belge De Med Tech 1952;32:173-8.

15. Wannisorn B, Jariksam S, Soontorntanasart T. Antifungal activity of lemon grass and lemon grass oil cream. Phytother Res 1996;10:551-4.

16. Sitara U, Niaz I, Naseem J, Sultana N. Antifungal effect of essential oil on in vitro growth of pathogenic fungi. Pak J Bot 2008;40:409-14.

Absence of anthelmintic activity of hydroalcoholic leaf extracts of Artabotrys hexapetalus (Linn.f)

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A B S T R A C T

Objective: *To study the anthelmintic activity of Artabotrys hexapetalus (Linn.f) leaves.*

Materials and Methods: *The extraction was made using the hydroalcohol with soxhlet extraction system. The hydroalcoholic extract of varying concentration was used for conducting anthelmintic activity (12.5, 25, 50 and 100 mg/ml). Eudrilus eugeniae (African adult earthworm) was used as the test organism.*

Result: *The time of paralysis and death of the earthworms, the positive control (albendazole) was more effective and no effect was shown by the hydroalcoholic extract of varying concentrations.*

Conclusion: *Hydroalcoholic extract of Artabotrys hexapetalus, didn't show any anthelmintic activity at the concentration of 12.5, 25, 50 and 100 mg/ml.*

Key words: Albendazole, Eudrilus eugeniae, hyroalcohol, soxhlet extraction

INTRODUCTION

Medicinal plants have served through ages, as a constant source of medicaments for the treatment of a variety of diseases. The history of herbal medicine is almost as old as human civilization.[1] The World Health Organization (WHO) estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the anthelmintic drug is sparse. According to the WHO, only a few drugs are used in the treatment of helminthes in humans.[2] Anthelmintics are the drugs or agents that destroy or cause the expulsion of parasitic intestinal worms. Helminthiasis is a macro parasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. It can have immunomodulatory effects on the host, with implications for any co-infecting pathogens.[3] An integrated approach is required for the effective control of helmintics, which includes strategic and tactical use of antihelmintics and careful management of grazing lands, including control of stocking rates and appropriate rotation strategies.[4]

Artabotrys hexapetalus (Linn.f) belongs to the custard apple family Annonaceae, consisting of trees

and shrubs with about 2300-2500 species and more than 130 genera. A. hexapetalus (Linn.f) commonly known as Manoranjini is a powerful climber. The old stems of great thickness are covered with rather smooth grey bark and furnished with thick woody pointed spreading spines 2-4 cm long. The leafy branches are slightly puberulous. The flowers are very fragrant with yellow petals and its fruit is narrowly obovoid. The bark and roots are in general, used for dysenteries and as vermifuges, and leaves for dysenteries and fevers. Traditionally, decoctions of the leaves are used as a remedy for cholera and have been found to exhibit antifertility effects in rats.

Despite the arrays of documented reports of A. hexapetalus (Linn.f), currently available literature revealed that there is a paucity of information on the potentials of this plant as a traditional remedy for intestinal helminthes.[5] As a part of research work efforts were made to investigate the in vitro anthelmintic activities of the hydroalcoholic extract of A. hexapetalus (Linn.f) leaves using adult African earthworm (Eudrilus eugeniae), which has similar anatomy and physiology to human intestinal helminthes.

MATERIALS AND METHODS

Collection and authentication of plant

The leaves of A. hexapetalus (Linn.f) maintained at Sugandhavana were collected from UAS (B), GKVK, Bangalore, Karnataka, India. The plant was identified and authenticated by Dr. M. Vasundhara, Professor, Division of Horticulutre, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka (No. 13/Hort/MADP [Authentication No. 1]).

Collection of worms

Worms required for evaluation of the anthelmentic activity of leaves of A. hexapetalus (Linn.f) were collected and authenticated by Prof. Govindraj, Department of Entomology, UAS (B), GKVK, Karnataka, India. Worms collected belonged to the genus E. eugeniae (African adult earthworm). The worms were placed in a ventilated bag with sufficient nutrients until the study was conducted.

Drugs and chemicals

In this study, albendazole was used as the Standard Drug (GlaxoSmithKline).[1] The concentration of standard drug was prepared in normal saline to give 15 mg/ml concentration. Normal saline was used during the experimental protocol.

Extraction process

Extraction is a process where the desired constituents of the plant are extracted using a solvent. The precise mode of extraction naturally depends on the texture and water content of the plant material

being extracted and the type of substance that is being isolated. Alcohol, in any case is a good all-purpose solvent for preliminary extraction. The classical chemical procedure for obtaining organic constituents from dry plant tissue (dried seeds, roots, and leaves) is through soxhlet apparatus using a wide range of solvents. The leaves were shade dried and made into coarse powder by using a mechanical grinder.

The powdered material was packed in soxhlet apparatus and extracted with 80% (v/v) ethanol. The extract was concentrated and dried. The dried hydroalcoholic extract of Artabotrys hexapetalus (HAAH) was stored in an air tight container in the refrigerator at <10°C.

Experimental model

Adult African earthworms (E. eugeniae) of 5-8 cm in length, 0.1-0.3 cm in width and weighing 0.8-4.0 g were used for all experimental protocol due to their general anatomical and physiological resemblance with the intestinal roundworms parasites of human beings.[6] All the earthworms were sourced from moist soil within GKVK University campus and washed with normal saline to remove all fecal and waste matters. They were authenticated at the Department of Biological Sciences, UAS, GKVK, Bangalore, Karnataka, India.

Anthelmentic activity

A. hexapetalus (Linn.f) leaf extract were prepared at varying concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml. A volume of 10 ml of each concentration of hydroalcoholic extract was delivered into a petridish. Then six worms (same type) were placed in it. Similarly, for each concentration of hydro alcoholic extract, six worms were used. Time for paralysis was noted when the worm did not revive even in normal saline. Time for death of worms were also recorded when the worms lost their motility followed by fading away of their body color (when dipped in warm water of 50°C). Albendazole (15 mg/ml in distilled water) was used as a positive control.[5]

RESULTS

Data given in Table 1 revealed that the hydroalcoholic leaf extract of A. hexapetalus (Linn.f) (HAAH) did not show anthelmintic activity at any of the tested concentrations. Considering the time of paralysis and death of earthworms, the positive control (albendazole 15 mg/ml) was more potent than HAAH. The control (distilled water with tween 80) did not show any activity against earthworms.

DISCUSSION

The hydroalcholic leaf extract of A. hexapetalus (Linn.f) does not possess vermicidal property, which has been confirmed by the treatment of various concentration of the extract. Since the season of harvest

might also have influenced leaves, which were harvested during the flowering season (February-April) there could be a possibility of translocation of metabolites from the source to the sink

Table 1: Anthelmentic activity of Artabotrys hexapetalus (Linn.f) leaf extract

Test group	Concentration mg/ml	Paralysis onset time (min)	Death time (min)
Control	-	-	-
Hydroalcoholic leaf extract of *Artabotrys hexapetalus (Linn.f)*	12.5	0	0
	25	0	0
	50	0	0
	100	0	0
Albendazole	15	6.15±0.02	53±0.08

that may be resulting in less concentration of phytochemical in the leaves.[7] Thus, it failed to display activity against the worms used in the study. Further research studies should be carried out using various species of organisms.

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REFERENCES

1. Sarojini N, Chakraborti CK, Das MD, Jaiswal P, Kumari SU. Anthelmintic activity of Clitoria Ternatea leaf extracts. J Pharm Res Opin 2012;6:49-50.

2. Ashok Kumar BS, Lakshman K, Jayaveera KN, Nandeesh R, Manoj B, Ranganayakulu D. Comparative in vitro anthelmintic activity of three plants from the Amaranthaceae family. Arch Biol Sci 2010;62:185-9.

3. Srilakshmi S, Sravanthi KC, Sarvani M, Krishnaharsha A, Karteek P. Anthelmintic activity of Annona squamosa seed extract. Int J Pharm Technol 2011;3:1623-8.

4. Akhtar MS, Iqbal Z, Khan MN, Lateef M. Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo–Pakistan subcontinent. Small Rumin Res 2000;38:99-107.

5. Ikechukwuogu G. In vitro anthelmintic potentials of Bambusa vulgaris (L.) Leaf extracts using adult African Earthworm (Eudrilus eugeniae) from Southern Nigeria. Indian J Novel Drug Deliv 2012;4:306-10.

6. Lakshmanan B, Mazumder PM, Sasmal D, Ganguly S, Jena SS. In vitro anthelmintic activity of some 1 substituted imidazole derivatives. Acta Parasitol Globalis 2011;2:01-05.

7. Yadav AK, Singh S, Dhyani D, Ahuja PS. Review on the improvement of Stevia [Stevia rebaudiana (Bertoni)]. Can J Plant Sci 2011;91:1-27.

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4. Scientific critique or forum (discussion on a particular scientific topic, based exclusively on management argumentation) and commentaries. Exceptionally, in particular areas, a scientific paper in the Journal can be in a form of a monograph or a critical edition of scientific data (historical, archival, lexicographic, bibliographic, data survey, etc.) which were unknown or hardly accessible for scientific research.

Professional articles:

1. Professional paper (contribution offering experience useful for improvement of professional practice but not necessarily based on scientific methods);
2. Informative contribution (editorial, commentary, etc.);
3. Review (of a book, software, case study, scientific event, etc.)

Language

The article should be in English. The grammar and style of the article should be of good quality. The systematized text should be without abbreviations (except standard ones). All measurements must be in SI units. The sequence of formulae is denoted in Arabic numerals in parentheses on the right-hand side.

Abstract and Summary

An abstract is a concise informative presentation of the article content for fast and accurate Evaluation of its relevance. It is both in the Editorial Office's and the author's best interest for an abstract to contain terms often used for indexing and article search. The abstract describes the purpose of the study and the methods, outlines the findings and state the conclusions. A 100- to 250- Word abstract should be placed between the title and the keywords with the body text to follow. Besides an abstract are advised to have a summary in English, at the end of the article, after the Reference list. The summary should be structured and long up to 1/10 of the article length (it is more extensive than the abstract).

Keywords

Keywords are terms or phrases showing adequately the article content for indexing and search purposes. They should be allocated heaving in mind widely accepted international sources (index, dictionary or thesaurus), such as the Web of Science keyword list for science in general. The higher their usage frequency is the better. Up to 10 keywords immediately follow the abstract and the summary, in respective languages.

Acknowledgements

The name and the number of the project or programmed within which the article was realized is given in a separate note at the bottom of the first page together with the name of the institution which financially supported the project or programmed.

Tables and Illustrations

All the captions should be in the original language as well as in English, together with the texts in illustrations if possible. Tables are typed in the same style as the text and are denoted by numerals at the top. Photographs and drawings, placed appropriately in the text, should be clear, precise and suitable for reproduction. Drawings should be created in Word or Corel.

Citation in the Text

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Footnotes

Footnotes are given at the bottom of the page with the text they refer to. They can contain less relevant details, additional explanations or used sources (e.g. scientific material, manuals). They cannot replace the cited literature.
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